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Insilico sequence analysis, homology modeling, molecular docking and functional annotation of peroxiredoxin from *Meloidogyne incognita*

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Abstract

Agricultural production in India mostly affected due to the parasitism of different pathogens, parasites and pests among them root knot nematodes (RKN) creating major problem in agricultural production which is directly shows effect on Indian economy. *Meloidogyne incognita* a nematode parasite which can infect almost 2000 different plant species. Peroxiredoxin is responsible for the defense response against plants and also responsible for causing nematode infestation in plants. It has been reported that Peroxiredoxin protein serves as a vital drug target to inhibit their growth and development. Inhibition of peroxiredoxin from nematodes may helpful to plants as it plays important role in plant defense mechanism. Current study aims to find phytochemicals which can be used as natural inhibitors of *M. incognita* by targeting Peroxiredoxin. To understand the structural and functional aspects of peroxiredoxin it is prerequisite to explore insights of protein primary, secondary structure and tertiary structure. In present study computational prediction of primary structure of Peroxiredoxin protein of *Meloidogyne incognita* is performed using Uniport. The physicochemical property analysis of peroxiredoxin protein were obtained from EXPASY server ProtParam. In secondary structure analysis amino acid composition of peroxiredoxin were predicted by PredictProtein. Three-dimensional structure of Peroxiredoxin from *M. incognita* were predicted by using Homology modeling approach three-dimensional structure of Peroxiredoxin was determined using protein structure prediction server (PS2 Server) selecting MODELLER as structure prediction method, predicted structure of protein also gets validated by using various validation tools. The AutoDock automated docking software were used for molecular docking and predicting optimal protein ligand interaction. Based on ADMET properties analysis and binding affinity all the three phytochemicals Withaferin A, Triptolide and Indole 3 Acetic acid shows nematocidal properties.

Keywords: *Meloidogyne incognita*, peroxiredoxin, homology modelling, three-dimensional structure, molecular docking

Introduction

Nematodes are the invertebrate roundworms which are very diverse in nature and inhabit in marine, freshwater and terrestrial environments etc. Among them Root knot nematodes creating major problem in agricultural production, which is result into decrease in agricultural production, most of specie of nematode attack on roots and feed on it. (Rajendra Singh, 2013) [1], Widely spread and dominant *Meloidogyne incognita* species mostly damage rice, potato, tomato crops in India and mostly found in monocotyledon host plant. (Phong Vunguyen, 2014) [2]. As a name suggest root knot nematode it shows knot and gall like structure at host plants root or tubers. These galls different in size from pin-head to generous size which in case of heavy infection may responsible to form large secondary galls. (M.R. Khan, 2014) [3]. Development and infestation of this parasite is completely depending on one of the specific gene which is called as PRX gene. When any parasite infects the plant, plant activate H2O2 mediates stress responses and the activates host defenses which kills pathogens, but ROS scavenging enzymes protect the parasite from oxidative response of the host tissue, Peroxiredoxins (PRXs) are Thiol-specific anti-oxidant protein that reduce H2O2 and alkyl hydroperoxide. Pathogenic nematode must fight with oxidative defense response of the plant so they have developed special defense mechanism to fight against plant defense response. Peroxiredoxin is responsible for this defense response against plant. Peroxiredoxin which responsible for severe nematode infestation in plant (Geraldine Dubreuil, 2010) [4]. So, to control the infestation of *M incognita* there is needs to target the important metabolic pathways and inhibit the synthesis of proteins/enzymes from parasite. Synthetic nematicides which is available in market inhibit the metabolic pathways of nematodes to control the spreads, but environment effective phytochemicals can be a better substitute for synthetic drugs, as they are available naturally and also shows not bad impacts on environment and hosts (Halder ST, 2019) [5]. Three important phytochemicals namely withaferin A from *Withania sominifera* (Qian Zhao, 2015) [6] Indole 3 Acetic acid from *Cerastium* (Anders O stin, 1998) [7] and triptolide from *Tripterygium wilfordii* Hook F (Anita M. Brinker, 2005) [8], which show nematocidal properties are screened and molecular docking infarctions with protein are analyzed. In the present study not only three-dimensional structure of Peroxiredoxin is predicted but also the molecular interactions between the protein and phytochemical ligands are analyzed which explains the promising nematocidal phytochemical to treat the root knot nematodes specially *M incognita*.

Materials and Methods

Protein Retrieval and Sequence Analysis- Protein sequence of Peroxiredoxin protein were retrieved from UniProt database with accession number [ACZ67203]. Prot Param tools was used to find out physicochemical properties of protein (Gasteiger E., 2005) [9]. Predict Protein analysis tool (Rost B, 2004) [10] were used for computing and analyzing the secondary structural features of peroxiredoxin in *Meloidogyne incognita* protein sequence. As three-dimensional structure of Peroxiredoxin is not available in biological databases and which is required to study the functional annotations. For three-dimensional structure prediction homology modeling-based approach followed by using MODELLER 9.24 tool (B. Webb, 2016) [11]. Based on maximum identity and lowest e-value, best suitable template with more than 80% identity was selected to generate protein model. The PROCHECK server (Laskowski R A, 1996) [12] of the PDBSum database (Laskowski R A J. J., 2017) [13] was used to predict, evaluate, and validate the homology build model of the Peroxiredoxin protein. The generated protein model was also validated by Ramachandran plot analysis. CASTp server were used to study surface features and functional regions of proteins, these include pockets located on protein surfaces and voids buried in the interior of proteins (Edelsbrunner H, 1994) [14] To inhibit the expression of Peroxiredoxin protein from nematode, molecules from phytochemicals namely, Withaferin A, Indole 3 Acetic acid and Triptolide were used. Preparation of ligand molecules was done after phytochemicals retrieved from the literature were drawn using Marvin Sketch and saved in. spdb format. Marvin Sketch of Chem Axon (Chem Axon Ltd., Budapest, Hungary) permits the user to speedily draw structure of molecules through basic functions on the graphical user interface and save them in many file formats (Chem Axon, 2017) [15] For verification the molecular structure of these ligand molecules was also retrieved from PubChem database (Bolton EE, 2011) ADMET analysis of selected ligand molecules were also performed by Swis ADMET server (A. Daina, 2017) [17] To observe the molecular interactions between protein and various ligands Molecular Docking was performed by using Auto Dock (Morris, 2009) [18] (Morris AL, 1992) [19] which is molecular modeling simulation software which is especially effective for protein-ligand docking.

Result and Discussion

Physicochemical Properties

To perform sequence analysis and molecular docking interactions of peroxiredoxin from *Meloidogyne incognita* the protein sequence was retrieved from Uniprot by using accession number ACZ67203. The physicochemical properties of peroxiredoxin protein shows that it contains 197 amino acids with Molecular Weight 22216.47 grams per mole. Isoelectric point (pI) the protein has no net charge because the positive and negative charges are equal Peroxiredoxin protein shows theoretical PI (isoelectric point) is 6.74. Peroxiredoxin is having Molecular formula of $C_{1006}H_{1565}N_{259}O_{294}S_{7}$. How much light a protein absorbs at a certain wavelength is indicated by the extinction coefficient. It is very essential to measure the coefficient for a protein with a spectrophotometer when purifying it, peroxiredoxin protein shows Extinction coefficients 21555Abs 0.1% (=1 g/l) 0.970. Analysis of half-life period is a calculation of the time it takes for half of the volume of protein in a cell to disappear after its formation in the cell target protein shows half-life of 30 hours (mammalian reticulocytes, *in vitro*) 20 hours (yeast, *in vivo*). 10 hours (Escherichia coli, *in vivo*).

Estimation of stability of peroxiredoxin protein in test tube i.e., instability index is 22.45. Any proteins whose shows the instability index smaller than 40 is projected as stable protein, so target protein classifies as the stable protein. The relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine) is defined as aliphatic index. It is regarded as a positive factor for the rise of thermostability of globular proteins peroxiredoxin shows aliphatic index 79.64. hydropathicity (plural hydropathicities). The Grand Average of Hydropathy (GRAVY) value for protein is -0.342, which is considered as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.

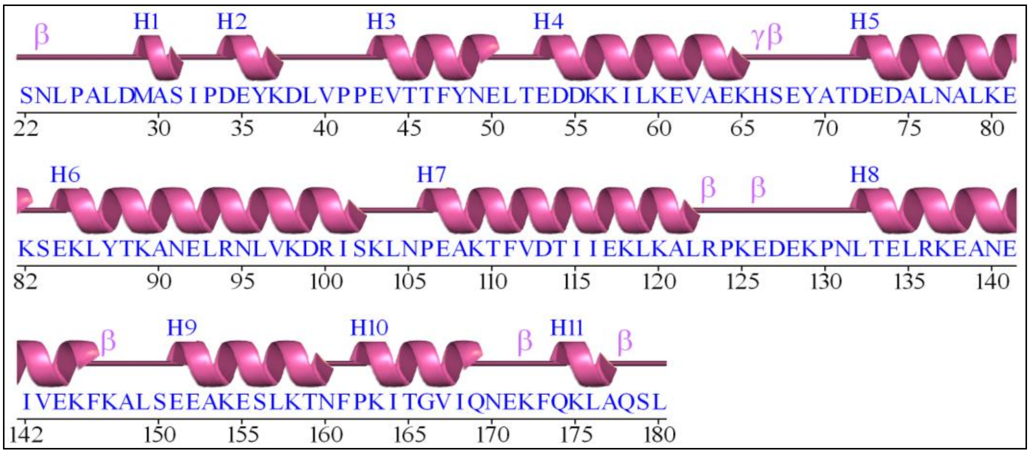


Fig 1: Secondary structure prediction of peroxiredoxin protein.

Secondary Structure Prediction

The PredictProtein analysis results of peroxiredoxin showed that protein is a mixed protein having composition of Helix =25.38%, strand = 18.27%, loop =56.36%, the solvent accessibility of peroxiredoxin in Meloidogyne incognita shows 48.73%, intermediate 11.17% and Exposed 40.10 %. Length of amino acid is 197, number of align protein in peroxiredoxin are 67, and number of matched PDB structure are 22. Fig 01

Three-Dimensional Structure Prediction and Validation Using Homology Modeling Approach.

Availability of three-dimensional structure of any protein is prerequisite to understand the functional annotations of protein The three-dimensional structure of peroxiredox in protein from *M incognita* were predicted using the homology modelling based approach as such structure is not available in related biological databases. Templates are selected on the basis of their sequence similarity with query sequence. PDB ID 1LML was selected for homology modeling which is an X-ray diffraction mode. Homology based MODELLER 9.24 tool were used to generate the three-dimensional structure of protein. Reliability of the predicted model was further checked by ERRAT which also analyzes the statistics of non-bonded interactions between different atom types and plots the values of the error function versus position of a residues sliding window, calculated by a comparison with statistics from highly refined structures (Fig 02). Further validation and confirmation of predicted three-dimensional protein structure is performed by Ramachandran plot analysis. Except those at the chain termini Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. In the Plot Glycine amino acid residues are separately identified by triangles as these are not constrained to the regions of the plot suitable to the other side chain forms. Different colors on the plot characterizes the different regions described in the dark colored areas in red resemble to the core regions representing the most favorable combinations of phi-psi values. For perfect model validation to have over 90% of the residues in these core regions, the ratio of residues in the core regions is one of the better guides to stereo chemical quality (Fig 03).

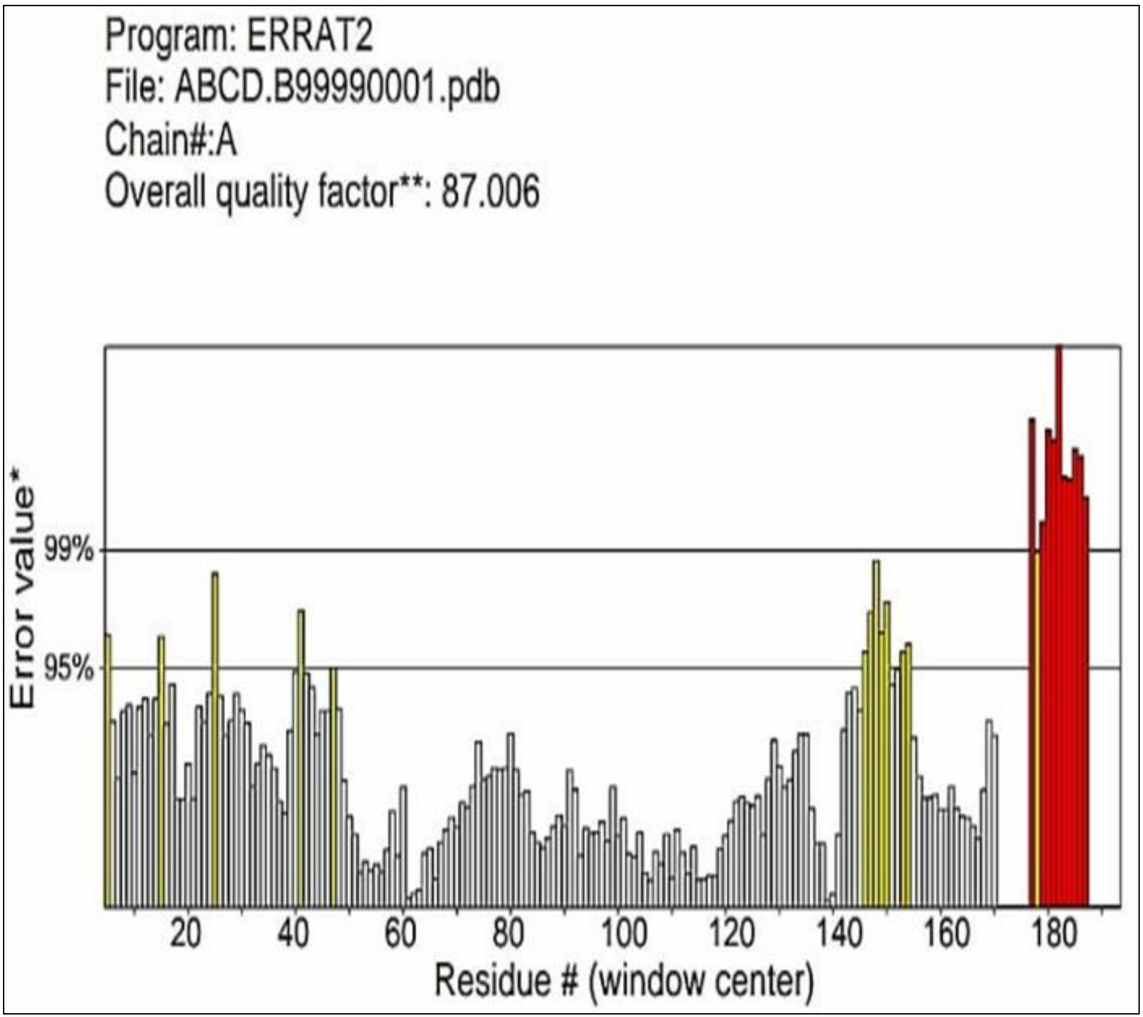
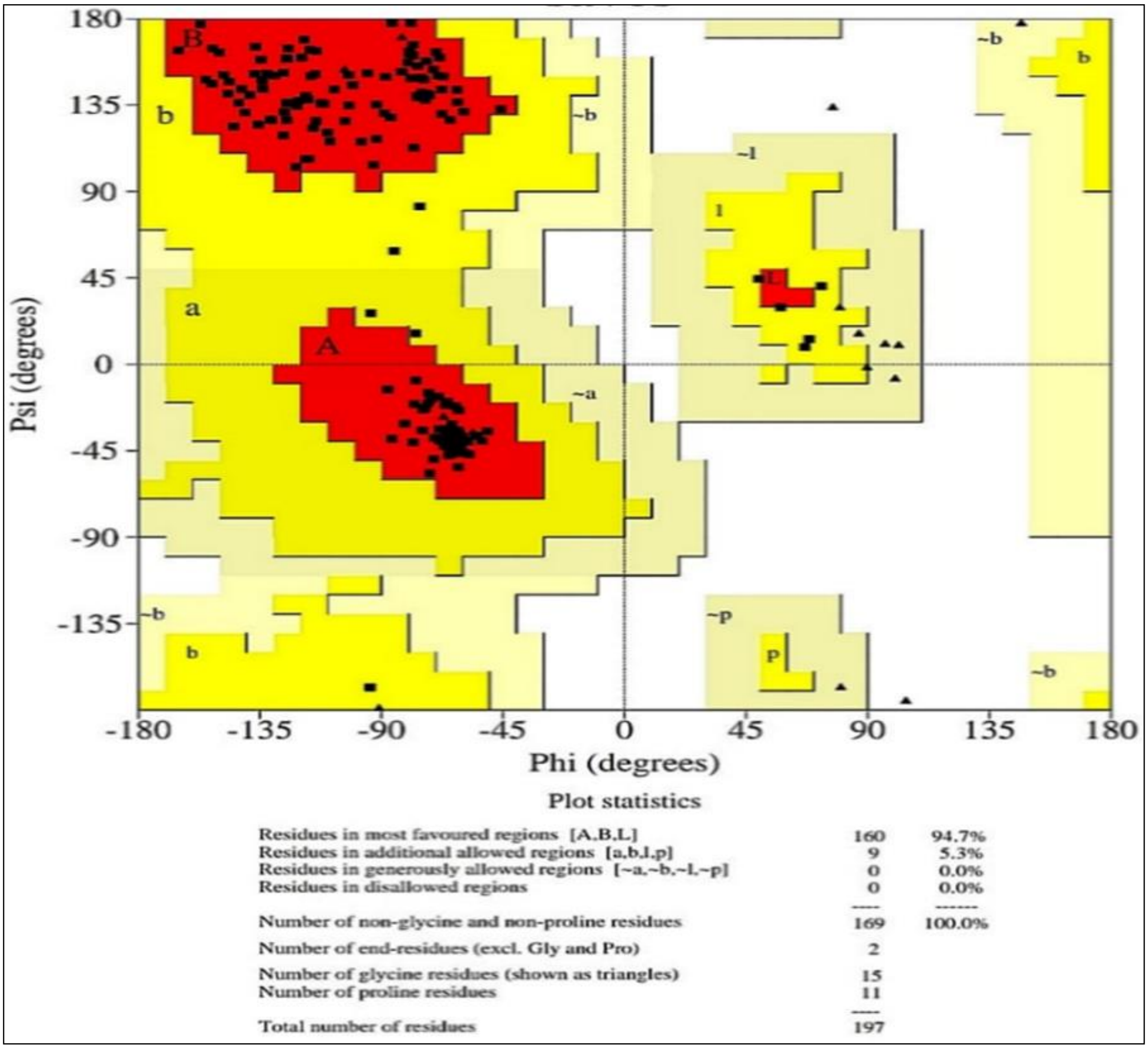


Fig 2: Overall quality factor of Peroxiredoxin from *M incognita* checked by ERRAT



Plot statistics

Residues in most favoured regions [A,B,L]	160	94.7%
Residues in additional allowed regions [a,b,l,p]	9	5.3%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	169	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	15	
Number of proline residues	11	
Total number of residues	197	

Fig 3: Ramachandran plot analysis of Peroxiredoxin

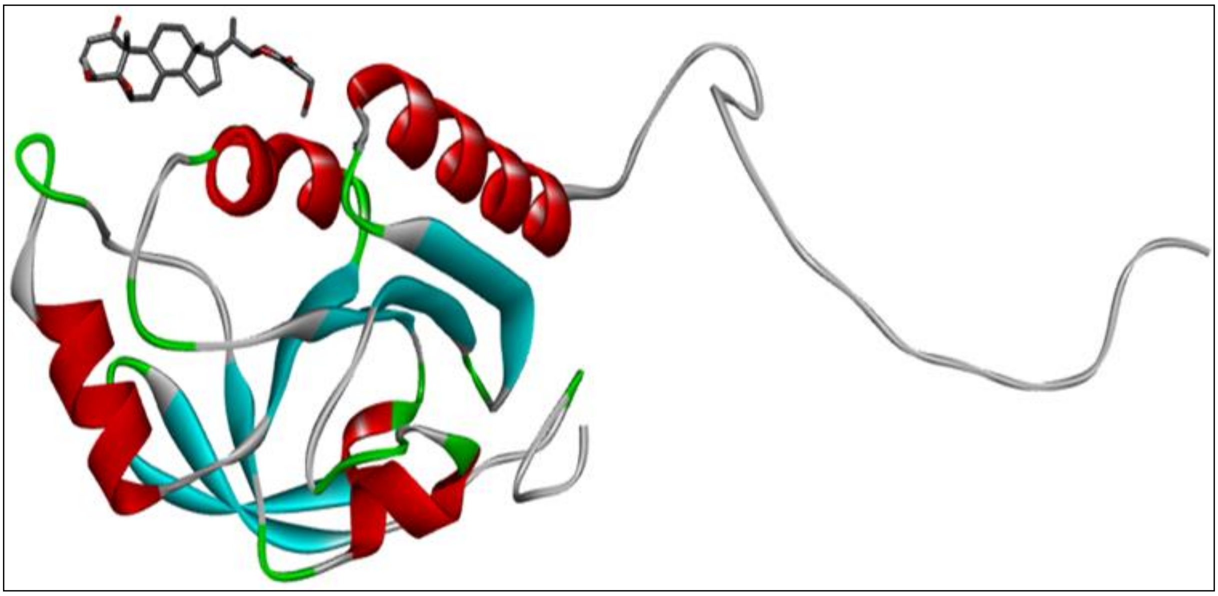


Fig 4: Homology modelling based predicted structure of Peroxiredoxin

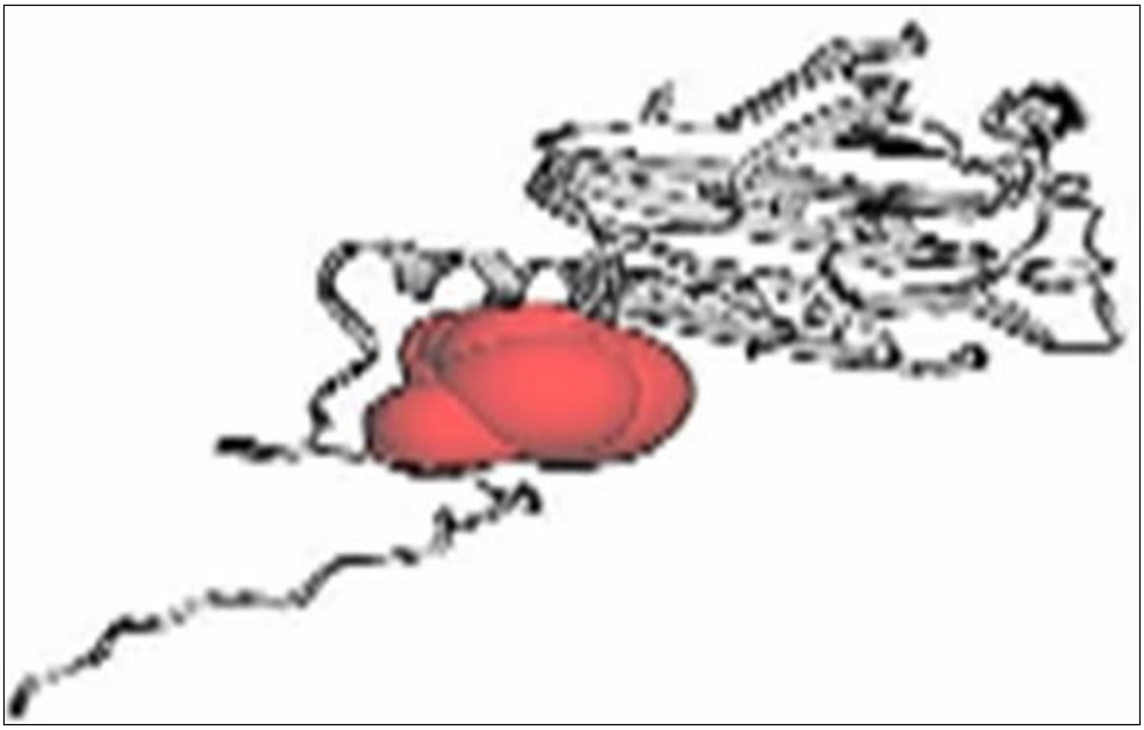


Fig 5: Active site pockets prediction by CASTp

Ramachandran plot shows amino acids in favored regions (A, B, L) 94.7% which is a proof that maximum amino acid residues are in favored region, residues in additional allowed region (a,b,l) 5.3%, and fortunately amino acid residues in disallowed region are 0.0%, that shows that our model is excellent quality and ready for further process.

Active site prediction and ADMET analysis by using CASTp and SwisADMET

The measurements and analysis of active sites of Peroxiredoxin protein include the area and volume of pocket or void by solvent accessible surface model, Theoretical Active site prediction of protein model was predicted using CASTP server, CASTP identifies and measures pockets and pocket mouth openings, as well as cavities, here we input the modeled protein for predicting the ligand binding sites and the CASTP server predicts the amino acids crucial for binding interactions (Fig 05).

Consensus Log Po/w values, water solubility, gastrointestinal absorption, drug-likeness, topological polar surface area in Å2 units, and Lipinski's rule of 5 predicted by SWISSADME server for 03 phytochemicals (Table 01)

Table 1: SWISSADMET analysis of ligands

Molecules	Swiss ADMET properties					
	Consensus Log $P_{o/w}$ values	**water solubility**	**gastrointestinal absorption**	**drug-likeness**	**TPSAÅ2 units**	**Lipinski's rule of 5**
Withaferin A	3.39	Moderately soluble	High	Yes	96.36 Å²	Pass
Triptolide	2.90	Soluble	High	Yes	84.12 Å²	Pass
Indole 3 Acetic acid	1.01	Soluble	High	Yes	53.09 Å²	Pass

Molecular Docking using AutoDock

InSilico Molecular docking analysis facilitates the valuable insights into the binding affinity and interaction of selected ligands with their target protein. The docking analysis helps to understand the receptor–ligand (protein–ligand) interactions at the molecular level. AutoDock allows to predict the binding affinity of the ligand with receptor protein for nine different ligand's conformational poses. The binding affinity and other conformational parameters like Number of Amino acids residues, Number of Hydrogen Bonds and Hydrophobic Bond Alkyl type interactions of various phytochemicals for their best conformational pose matches is highlighted. The binding affinity of ligands to target protein predicted by AutoDock helps us in determining which selected ligand has stable protein–ligand interaction with the target protein. The binding affinities of phytochemicals withaferin A from *Withania sominifera*, shows the binding affinity of –6.3 kcl/mol, Indole 3 Acetic acid from *Cerastium* shows the binding affinity –6.1 kcl/mol, triptolide from *Tripterygium wilfordii* Hook F, shows binding affinity –6.2, From all the three selected phytochemicals Withaferin A showed the binding affinity -6.3 kcl/mol with 04 interaction (Fig 06) 1) ILE56-UNL1 both Amino acids formed Hydrophobic bond distance of bond is 5.22909 that bond is Alkyl type 2) ALA57-UNL1 Amino Acid formed Hydrophobic bond, and it is Alkyl type distance of bond is 5.10684 3) A: ALA57 -: UNL1:C both formed Hydrophobic bond which is alkyl type distance of bond 4.19166 4):UNL1:C - A:ARG61 this amino acids also formed hydrophobic bond which is alkyl type, distance of bond is 4.85921 5):UNL1:C - A:VAL152 this last bond, it is also hydrophobic and alkyl, distance of bond is 3.96192 (Table 02).

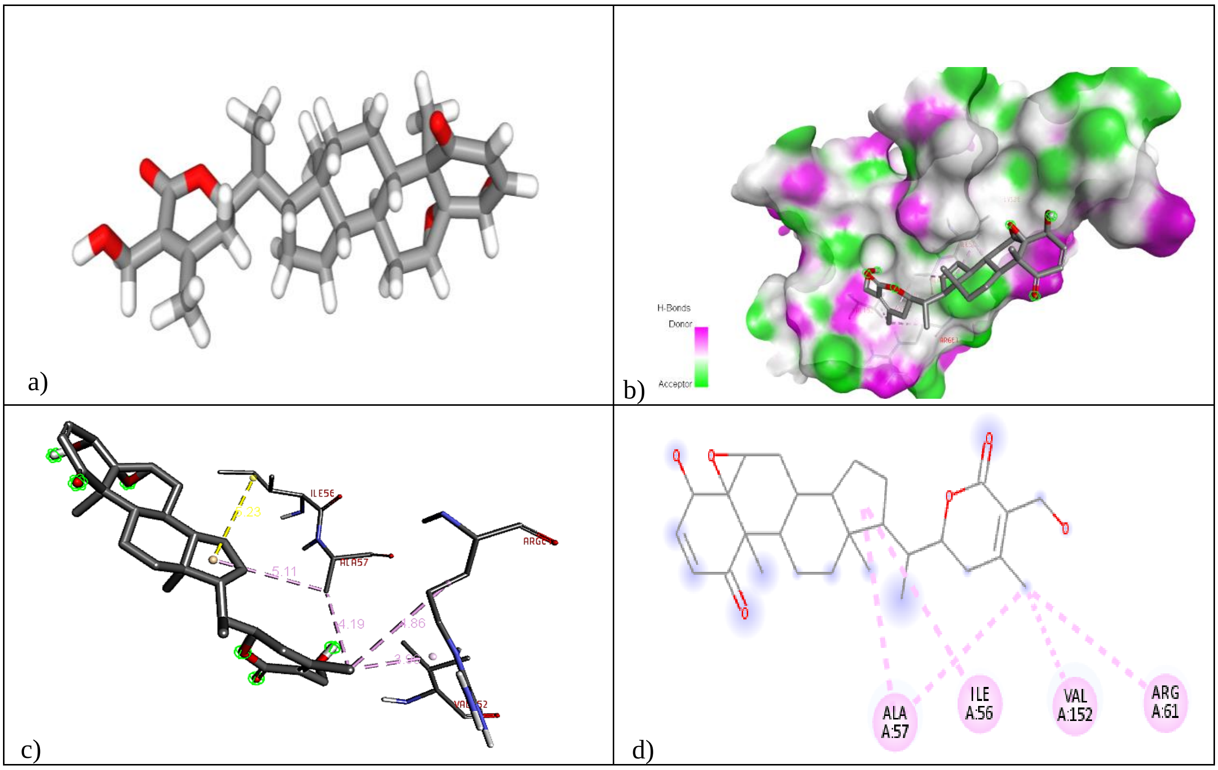
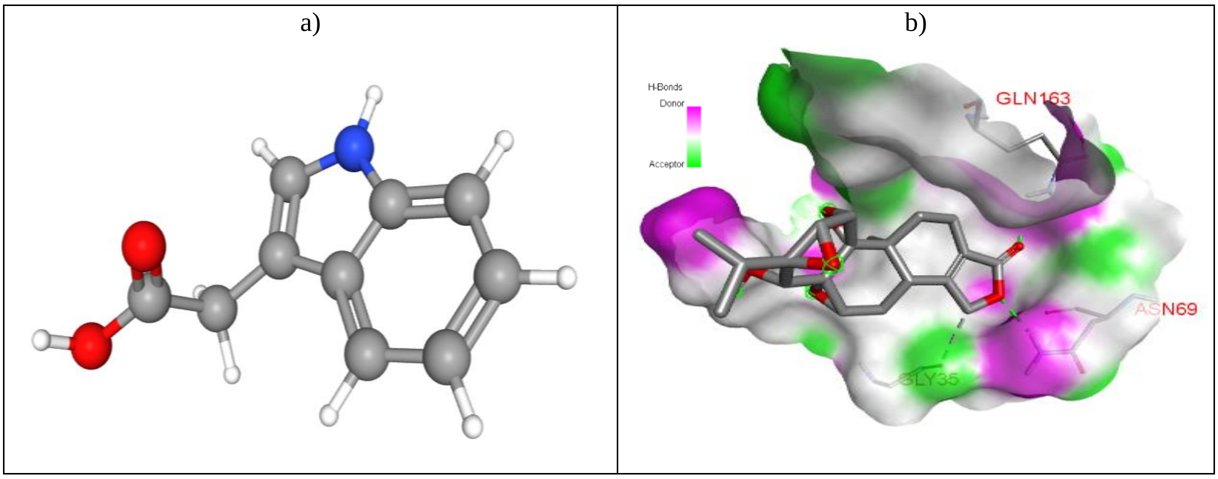


Fig 6: Output of AutoDock visualized using Discovery Studio Visualizer showing interacting binding-site residues a) Ligand Withaferin A b) and c) Interactions between Peroxiredoxin and Withaferin A, interacting binding sites are labeled with colors and d) 2D diagram showing types of interaction formed between Withaferin A and Peroxiredoxin.

Peroxiredoxin and Indole-3-acetic acid interactions shows there are three Hydrogen bonds interactions (Fig 07) which are 1) A: ASN69:HD21 -: UNL1:O- - conventional Hydrogen Bond, A: ASN69 is H- donor and UNL1-H- acceptor with distance 2.11015. 2) A: GLN163:HE21 -: UNL1:O- conventional Hydrogen Bond A: GLN163 – H-donor UNL1:O - H acceptor with Distance 2.34671) 3): UNL1:C - A: GLY35 Carbon hydrogen bond UNL1:C = H- Donor A: GLY35= H-Acceptor with distance of 3.57387.



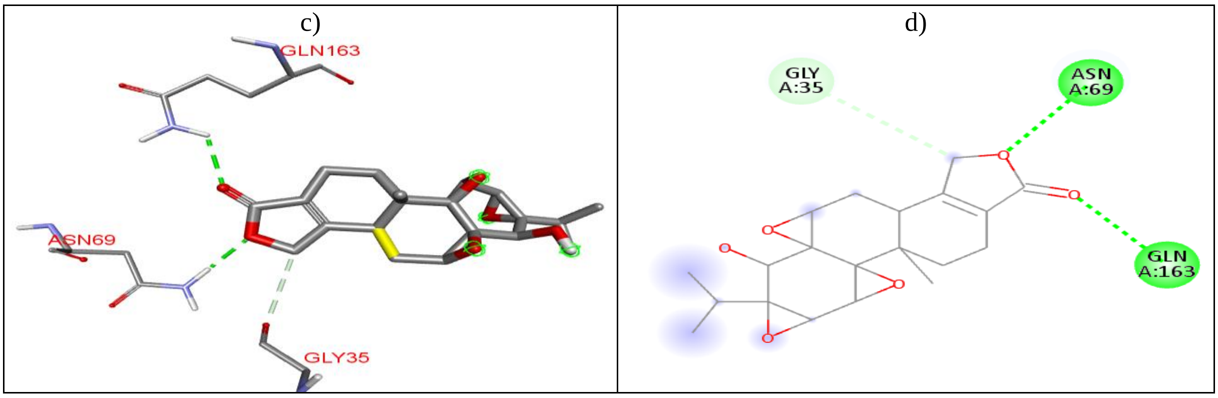

c) d)

Fig 7: Output of AutoDock visualized using Discovery Studio Visualizer showing interacting binding-site residues a) Ligand Indole-3-acetic acid b) and c) Interactions between Peroxiredoxin and Indole-3-acetic acid, interacting binding sites are labeled with colors and d) 2D diagram showing types of interaction formed between Indole-3-acetic acid and Peroxiredoxin.

Tripterygium wilfordii roots have a long history of use in traditional Chinese medicine, The major active component of Tripterygium root extracts is the diterpenoid triptolide. There are three (Fig 08) conventional Hydrogen bonds,1) A: ASN69:HD21 -: UNL1: O 2) A: LYS167: CE -: UNL1:O 3): UNL1:C - A: ASP166:OD2 all bonds are conventional type hydrogen bond, distance between A: ASN69:HD21 -: UNL1: O is 2.12027, in there interaction A:ASN69:HD21 is H-Donor and:UNL1: O is H-Acceptor, second bond UNL1:H -: UNL1: O is showing distance 2.86255 in that interaction:UNL1:H is H-Donor And:UNL1: O H-acceptor, A: LYS167: CE -: UNL1: O shows distance 3.34431 that interaction A:LYS167:CE is H-donor and:UNL1:O is H-acceptor'. Last bond UNL1:C - A: ASP166:OD2 shows maximum distance 3.56302 and: UNL1:C is H- donor, A: ASP166:OD2

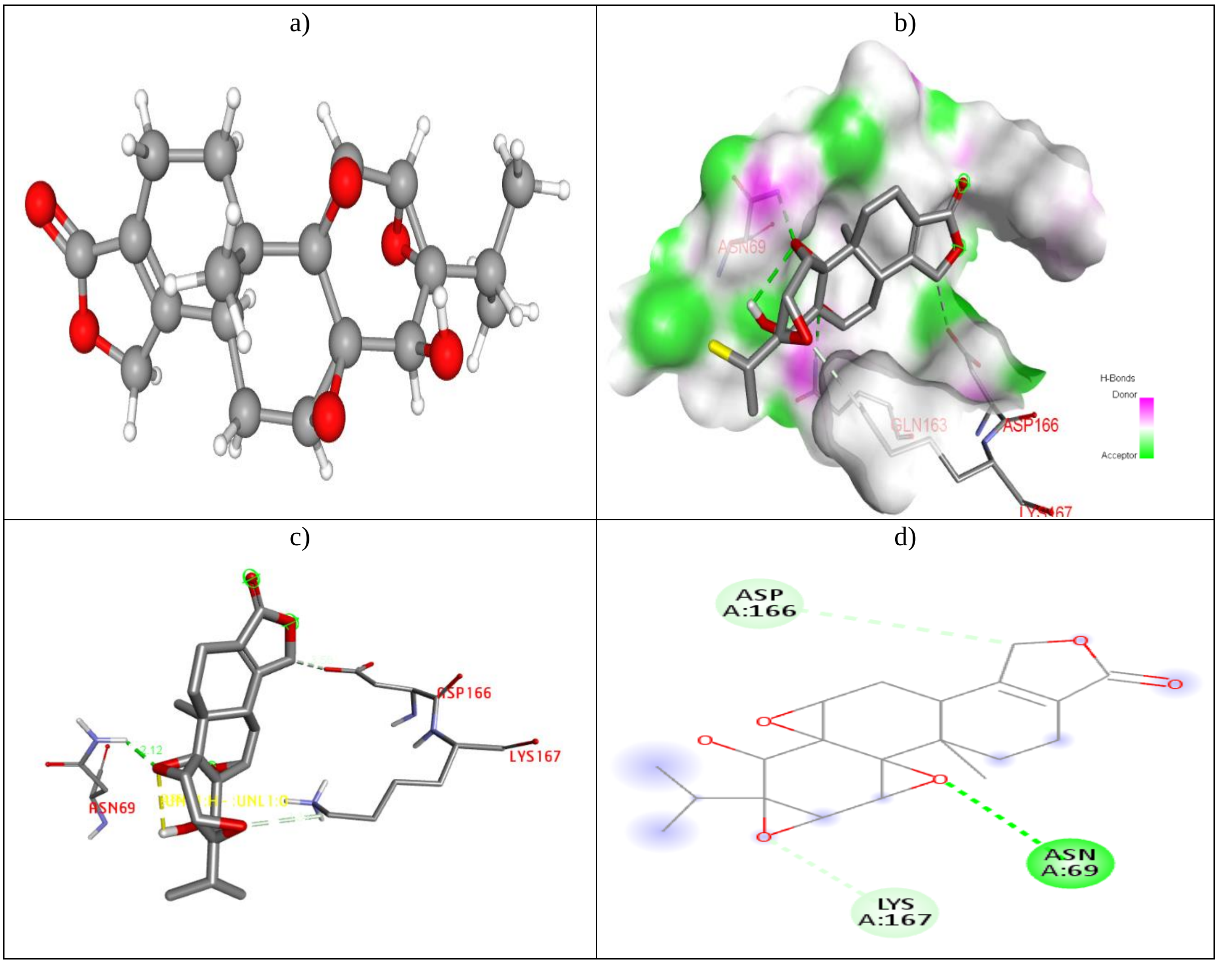

a) b) c) d)

Fig 8: Output of AutoDock visualized using Discovery Studio Visualizer showing interacting binding-site residues a) Ligand triptolide b) and c) Interactions between Peroxiredoxin and triptolide, interacting binding sites are labeled with colors and d) 2D diagram showing types of interaction formed between triptolide and Peroxiredoxin.

Table 2: Comparison of binding affinity (kcal/mol) of phytochemicals for targeted protein.

Name of the ligand	Withaferin A	Triptolide	Indole 3 Acetic acid
Affinity (Kcal/mol)	-6.3	-6.2	-6.1
Number of Amino acids residues	4	3	3
Number of Hydrogen Bonds	0	3	3
Hydrophobic Biond Alkyl type	4	0	0

Conclusion

The current study focused on the prediction of three-dimensional structure of peroxiredoxin protein and screening of phytochemicals for inhibition of nematodes *M incognita* which cause severe damage in most of the plants by damaging the roots and forming knots. Peroxiredoxin protein from RKN *M incognita* is responsible for development of Nematodes. Along with primary and secondary structure analysis, the three-dimensional structure of target protein is prerequisite for structural and functional analysis. Comparative computational molecular modelling and molecular docking analysis of Peroxiredoxin and phytochemicals showed that all the three ligands show almost similar activity of inhibition with slight variations (Table 02). All the three phytochemicals pass the ADMET analysis and shows the drugability.

Withaferin A from *Withania sominifera*, shows the binding affinity of –6.3 kcl/mol, Indole 3 Acetic acid from *Cerastium* shows the binding affinity –6.2 kcl/mol, triptolide from *Tripterygium wilfordii* Hook F, shows binding affinity –6.2, as above results shows these are the promising candidates to suppress the expression of peroxiredoxin from nematode *Meloidogyne incognita*, and they may block Oxygen metabolism, cell signaling process and hemostasis. For confirmation of above results *in vivo* and *in vitro* findings must be essential.

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