IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-14, Issue3, 2023, pp01-10
www.bipublication.com

Research Article

Insilico Docking Studies of Natural Bio active Compounds against Chitin Synthase of Magnaportheoryzae reveals Anonine and Nimboline-B as strong Anti-fungal agents.

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[Received 08 June-2023, Modified 01 July-2023, Accepted 18 July-2023, Published 26 July-2023]

Abstract

Magnaporthe oryzae also known as Rice blast fungi is most prevalent rice fungal Disease across rice growing regions of the world. Among all the factors resulting in reduced yield in rice, infection with rice blast fungus is a significant factor affecting the yield. Hexaconazole is widely used fungicide for controlling *Magnaporthe oryzae*, however alternative natural molecules which acts as good fungicide against this disease is the need of the hour considering potential harmful effects of the synthetic molecules. Insilico repurposing is a technique to discover potential molecules against diseases and it also cuts Research and Development cost and time. Chitin Synthase is present in phospholipidbilayer of fungal cell membrane.

It is mainly responsible for the production of Lipo polysaccharides in the fungal cell wall. Blocking of Chitin Synthase will disrupt the formation of fungal cell wall which makes it a potential candidate for targeting.Chitin synthase of *Magnaporthe oryzae* amino acid sequence was taken from uniprot database ID CHS4_MAGO7and a 3-D model was downloaded from the Alphafold Server. The model was validated by using Ramachandran plot.

Quality of the protein sequenced was validated by using ERRAT software. Glob plot analysis was done to identify conserved domains which revealed the presence of TransMembrane region and Cytochrome B 450. After docking studies we conclude that two bio active compounds Anonaine from *Annonasquasoma* and Nimboline - B from *Azadirachta indica* out of nine studied compounds shows strongest binding affinity to chitin syntahse of *Magnaportheoryzae*. Further molecular dynamics studies followed by bioassays will help in obtaining a strong molecule for control of *Magnaportheoryzae*.

Keywords: Chitin Synthase, Insilico Molecular Docking, *Magnaportheoryzae*, Anonaine, Nimboline – B, Modelling.

Introduction

Bio pesticides and Bio fertilizers are going to be used extensively because of their many advantages to agriculture and environment. Now a day's farmers are commonly using chemical fertilizers to attain maximal biomass and yield [1].The management of insect pests and fungal diseases that cause damage to crops has become challenging due to the rise of pesticide and fungicide resistance [2]Plant-derived compounds are regarded as a substantial source for novel lead structures to develop new natural bio pesticides products. Concurrent with greater awareness towards the use of synthetic chemicals in agricultural practice, the application of integrated pest management programs has also increased. In recent years, there has been considerable public pressure to reduce the use of synthetic fungicides in agriculture. Although, the use of synthetic fungicides in plant disease control has been successful in improving agricultural output, several of these have been found to exhibit side effects in the form of resistance and residual toxicity. The alternative green choice therefore would be the use of bio fungicides and bio pesticides, which are found to be largely nonphytotoxic, systematic and easily biodegradable in nature [3]).

Application of bio-inoculants to the soil would increase the symbiotic relationship of the plants with the microorganisms which are having immense benefits. These bio-inoculants are very beneficial due to their positive effect on plant nutrient uptake from soil [4]. Bioactive compounds affect the fungi via interference with molecular targets in their organs, tissues and cells. The major targets include: Bio membrane, proteins and nucleic acids. Bioactive compounds are still regarded as a valuable pool for discovering novel mode of action [5]However, minimal studies have been conducted on assessing the chemical properties and nutrient contents of these fermented concoctions which led to enhanced plant growth [6]. Neem (*Azadirachta indica)* is a most versatile and useful medicinal plant. Its every part is rich in bioactive compounds, which have traditionally been used to treat different ailments including infectious diseases. Bioactive compounds such as Nimboline, Nimbin, Nimbolin- B and Azadirachtin of neem are reported to have a tremendous ability to regulate numerous biological processes in vitro and in vivo. Also studies have shown that there are other plant bioactive compounds which play crucial role in the disease resistance in plants derived from *Annonasquamosa*, *Annonamuricata*, which are Reticuline Annoine, Nornuciferine, Corypalmine and Asimilobine [7]. Curcumin extracted from the rhizomes of *Curcuma longa* shows strong antifungal activity[8].

Rice cultivation is a major activity and source of income for millions of households around the world [9]. Between 10% and 30% of the annual rice harvest is lost due to infection by the rice blast fungus *Magnaportheoryzae* causing a significant economic loss to the farmer. It is estimated that the rice which is sufficient to feed 60 million people, is being lost due to the infection from the rice blast fungus [10].

Chitin is an essential part of the carbohydrate skeleton of the fungal cell wall and is a molecule that is not represented in humans and other vertebrates. The 3D network of chitin micro fibrils is covalently attached to β(1,3)-glucan, second load-bearing polysaccharide present in most fungal cell walls. Chitin is synthesised by large families of chitin synthases (CHS) enzymes that fall into seven classes. In most fungi, chitin and cell wall synthesis occurs at sites of polarized growth [11]. During early bud growth, cell wall material is deposited at the bud tip[12]. Disruption of cell wall biosynthetic genes or treatments with cell wall perturbing agents often results in activation of chitin synthesis, in an attempt to maintain cellular integrity [13]. The fungal cell wall is a dynamic structure that protects the cell from changes in osmotic pressure and other environmental stresses, while allowing the fungal cell to

interact with its environment. The structure and biosynthesis of a fungal cell wall is unique to the fungi, and is therefore an excellent target for the development of anti-fungal drugs[14].Computational pharmacology and industrial drug designing's using insilico techniques to better understand and predict how drugs affect biological systems, which can improve clinical use, avoid unwanted side effects, and guide accurate selection and development of better treatments. One exciting application of computational pharmacology is drug repurposing. While current techniques in computational pharmacology and drug repurposing often focus on just a single data modality such as gene expression or drug–target interactions, the study of how two or more molecular structures e.g., drug and enzyme or protein fit together is done by docking studies[15]. In a docking, molecular modeling technique is used to predict how a protein-enzyme interacts with small molecules -ligands. The ability of a protein (enzyme) and nucleic acid to interact with small molecules to form a supermolecular complex plays a major role in the dynamics of the protein, which may enhance or inhibit its biological function. Based on the types of ligands the molecular docking is determined as 1.Protein- ligand docking 2.Protein- nucleic acid docking 3.Protein-protein docking [17]. The present study is a summary which focused oninsilico docking analysisof bioactive compounds isolated from plants against plant pathogenic fungi cell wall component.

Materials and Methodology

Modeling of Chitin Synthase

Homology modelled three dimensional (3D) structure of Chitin Synthase was downloaded from the Deep Mind Algorithm alpha fold system (https://deepmind.com). Chitin Synthase sequence of *Magnaportheoryzae* of rice blast disease from Uniprot(KB-IDCHS4_MAGO7) was used as an input for the construction of the 3D model. The Alpha Fold System from Deep mind uses conventional neural network for protein structure prediction[16]. Procheck, Errata analysis was done to confirm the 3-D structure of downloaded Chitin Synthase and to verify its quality factors and sequence alignment. CastP3.0 server was used to confirm the active sites of Chitin Synthase. Finally after confirmations, the downloaded model was 3D visualizedby using PyMOL.

Ligand selection and preparation

Structure-based virtual screening plays an important role in drug discovery and complements other screening approaches. In general, protein crystal structures are prepared prior to docking in order to add hydrogen atoms, optimize hydrogen bonds, remove atomic clashes, and perform other operations that are not part of the x-ray crystal structure refinement process[18].Dictionary of chemical components (ligands, small molecules and monomers) referred in PUBCHEM[19].Entries provide comprehensive search facilities for finding a particular component, or determining components in structure entries or vice versa. Many natural concoctions are used by the farmers of South India which contain use of various leaves as a composition. Most commonly used plant leaves are from Neem, Custard Apple and rhizomes of Turmeric. The leaves of Neem contain bio compounds Azadirachtin, Nimbin and Nimbolin-b. Anonaine, Nornuciferine, Corypalmine, Asimilobineand Reticuline are bio compounds commonly found in Custard Apple whereas,Curcumin is found in Turmeric rhizomes. These nine naturally occurring compounds which are widely used in the natural concoctions were selected to check their inhibiting capacity of Chitin Synthase of *Magnaporthe oryzae* using insilico Molecular docking strategy. All the 3-D structures of these nine natural compounds were downloaded from pubchem database and saved in PDB format (Fig 1). The Molecular docking was performed by using the selected ten natural compounds against chitin synthase of *Magnaportheoryzae.*

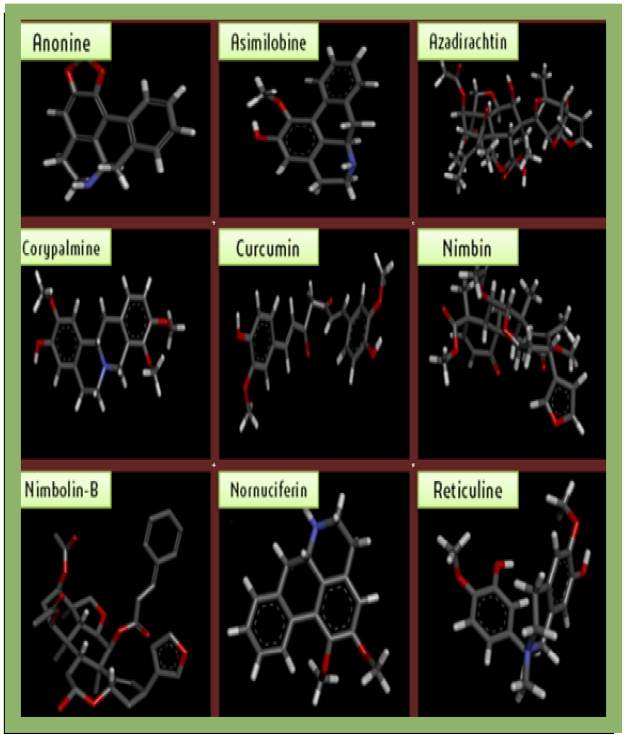

Fig: 1 3D structures of ligands from pubchem database Molecular docking

In a simple definition, docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands). The ability of a protein (enzyme) and nucleic acid to interact with small molecules to form a supramolecular complex plays a major role in the dynamics of the protein, which may enhance or inhibit its biological function[20].To carry out the molecular docking studies, virtual screening software PYRX was used. PYRX works based on empirical –based free energy scoring and Lamarckian genetic algorithm[7] Fig 2).All the ligands and macro molecules are converted from PDB format to PDBQT format by choosing auto dock option in PYRX. Molecular docking was performed in the grid box generated as X, Y, and Z axis and dimensions were adjusted to X; -1.2787 Å Y; 5.6948 Å X; 2.8507 Å based on the binding site with an exhaustiveness of 8 for blind docking, and the calculations were conducted in such a manner that only lowest energy pose was obtained as an output. The Docking results were analyzed on the basis of binding energy scores. All ligands displayed acceptable binding energy score.

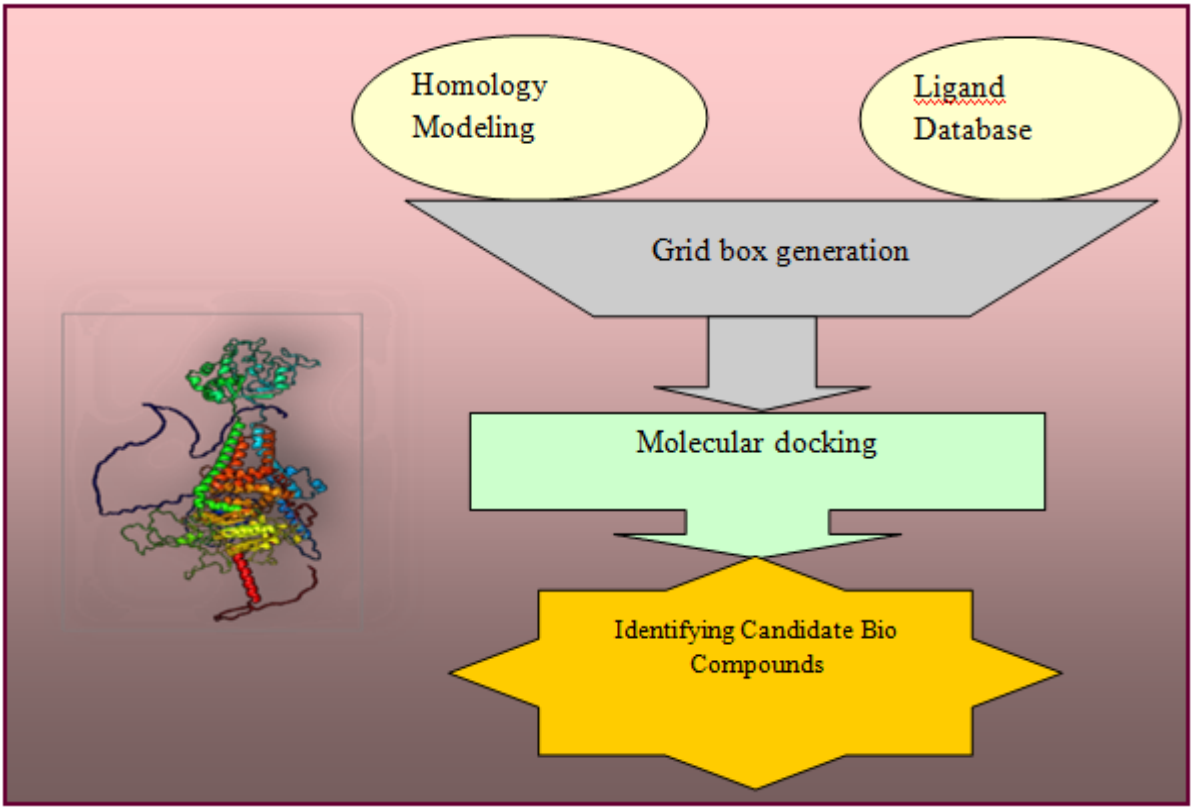

Fig: 2. Schematic representation of the workflow implemented in the current study

RESULTS

The present study provides comprehensive details on targeting fungal cell wall component Chitin synthase using homology modeling, molecular docking based virtual screening and free energy calculations. Homology modelled structure of chitin synthase was downloaded from Deep Mind algorithm Alpha Fold server systems. The server uses deep neural network learning algorithm (https://deepmind.com/). Chitin synthase protein sequence (UniprotKB id- KB- ID CHS4_MAGO7) was used as input for the construction of 3D model(Abraham et al.,2021). Below is the chitin synthase sequence developed using Alpha Fold modelled database for further docking studies (Fig. 3). Globe plot analysis of Chitin Synthase revealed the presence of 4 trans membrane domains in the regions 241-263; 1061-1083; 1090-1112; 1117-1139 (Fig.4). The presence of Trans membrane domains indicates that the enzyme is predominantly present in the fungal membrane as its role is in the synthesis of Chitin.

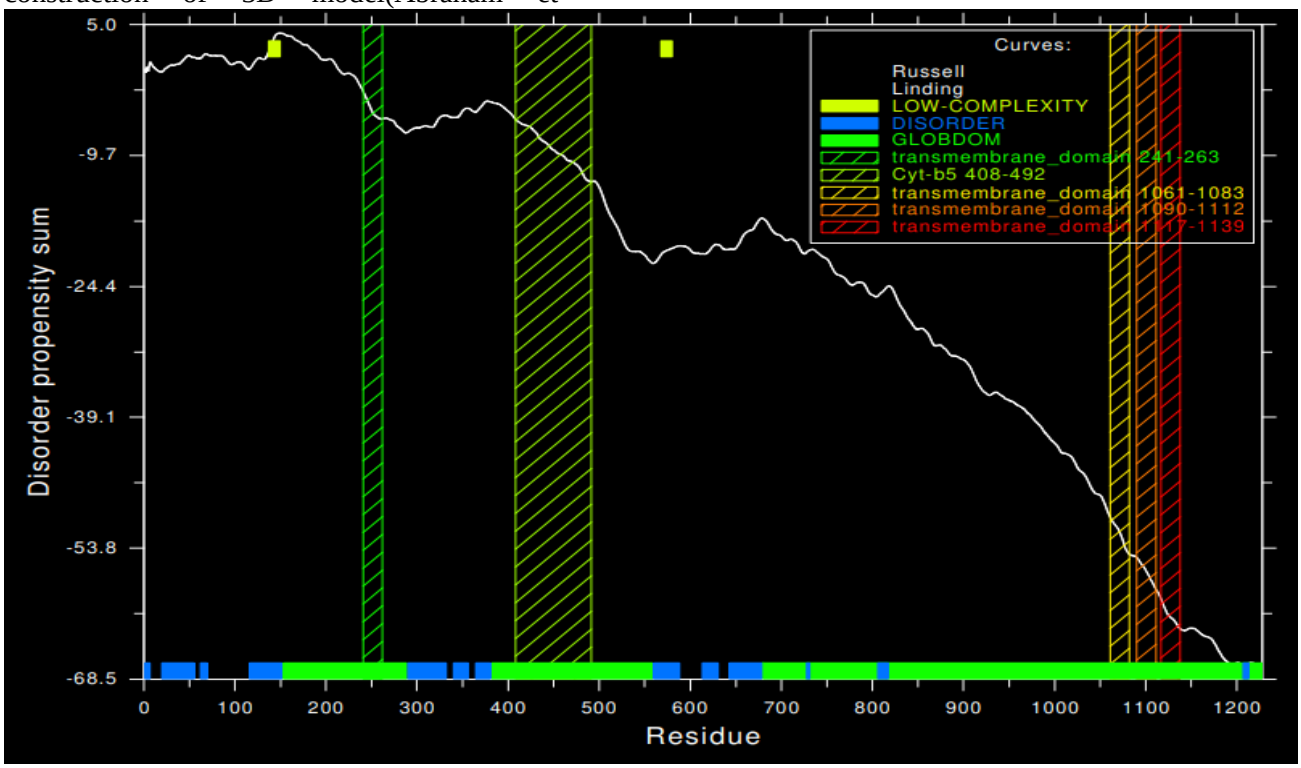

Fig. 3 Globplot analysis of Chitin Synthase

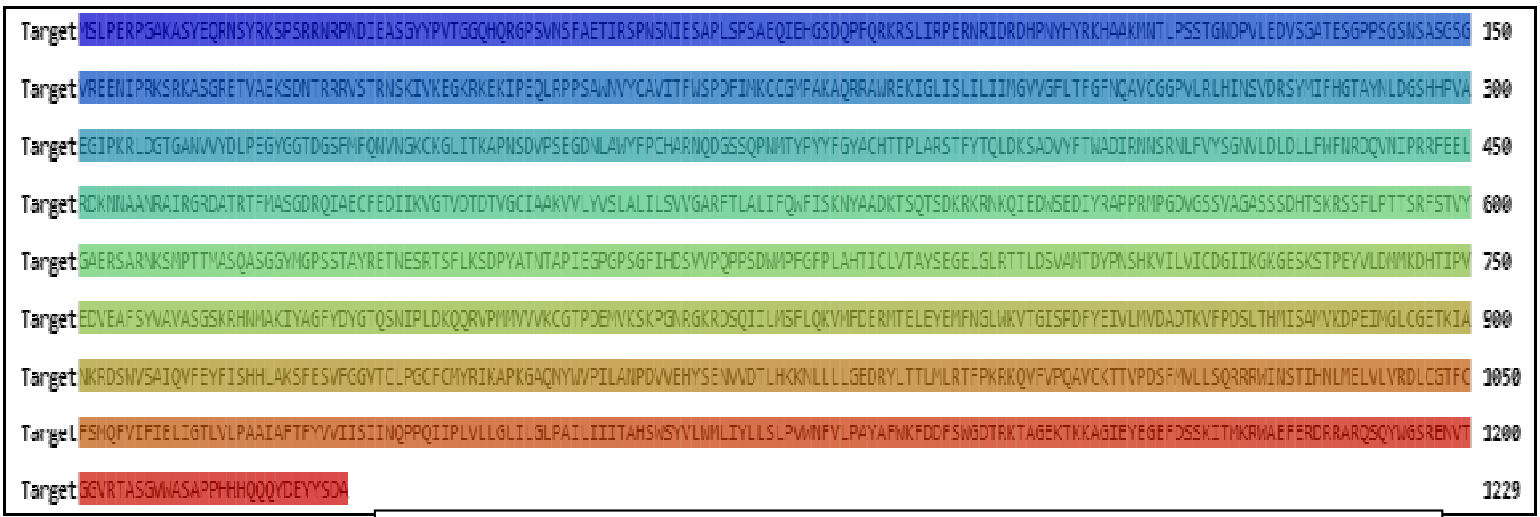

Fig. 4 Chitin synthase sequence of *Magnaportheoryzae* from Uniprot

Molecular docking of bio active compounds from *Annonasquamosa*reveals specific binding patterns

The behavior of small molecules in the binding pockets of target proteins can be described by molecular docking. To carry out the molecular docking studies, virtual screening software PYRX was used. PYRX works based on the empirical binding, free energy scoring and Lamarckian genetic algorithm. All the ligands and macro molecules are converted from PDB to PDBqt format by choosing auto dock option in PYRX. Molecular docking was performed as in the grid box generated in X, Y and Z axis based on binding site information (Fig. 5).

The Bio Active compoundsfound in *Annona squamosa* -Annonaine, Asimilobine, corypalmine, Nornuciferine, and Reticuline were docked with chitin synthase in the grid box generated based on the active site information provided by the castp3.0 online server. Anonaine showed interaction with MET 283, MET 330, VAL 314, PHE 331, HIS 387, ALA 392 of chitin synthase with a docking score of -8.5kcal/mol. Asimilobine showed interaction with MET 615, TYR 953, TRP 954, PHE 641, MET 833 of chitin synthase with a docking score of -7.4kcal/mol. Corypalmine showed interaction with ILE 1162, LYS 978, PHE 144, ALA 236, ALA 1160, ASP 1143, LYS 1159 WITH A DOCKING SCORE of -7.5kcal/mol. Nornuciferine showed interaction with TRP1140, ARG1150, TYR 692, LYS 818 of chitin synthase with a docking score of -7.6 kcal/mol. Reticuline showed interaction with ARG1025, ILE 1029, ILE1033, ARG 987, PRO 936, CYS 934,GLU 914, TYP 915, HIS 919 with a docking score of -7.0 kcal/mol.

Among the five natural compounds from *Annona squamosa*, Anonaine with a binding score of -8.5kcal/mol showed strongest interaction with chitin synthase protein in insilico docking studies (Table 1). The active sites of this protein –ligand binding structures were given below in figure5

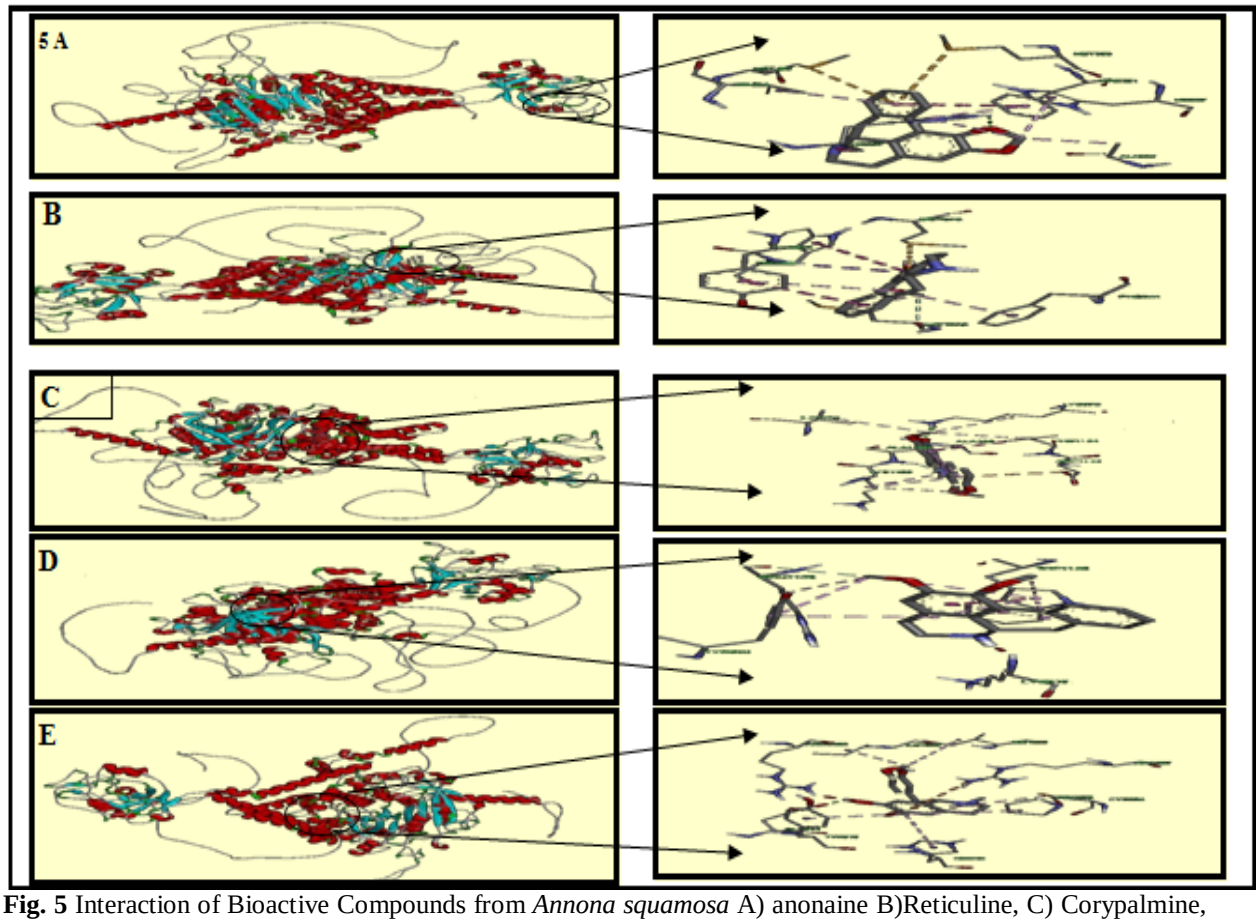

Fig. 5 Interaction of Bioactive Compounds from *Annona squamosa* A) anonaine B)Reticuline, C) Corypalmine, and D)NornuciferineE) Asimilobine with Chitin Synthase

S.No	Enzymes/Bio Compound	Interacting amino acids / Pocket amino acids	Docking Scorekcal/mol

01	**Anonaine**	**MET 283, MET 330, VAL 314, PHE 331, HIS 297, HIS 387, ALA 392**	**-8.5**
02	Asimilobine	MET 615, TYR 953, TRP 954, PHE 641, MET 833	-7.4
03	Corypalmine	ILE 1162, LYS 978, PHE 1144, ALA 236, ALA 1160,ASP 1143, LYS 1159	-7.5
04	Nornuciferine	TRP 1140, ARG 1150, TYR 692, LYS 818.	-7.6
05	Reticuline	ARG 1025, ILE 1029, ILE 1033, ARG987, PRO 936, CYS 934, GLU 914 TYR 915, HIS 919.	-7.0

Table. 1 *Annonasquamosa* interacting amino acids and molecular docking score

Nimbolin-B a bioactive compound from *Azadirachtaindica*shows strong affinity to chitin synthase in insilico analysis

Bio active compounds from *Azadirachtaindica* have been well documented for its pharmacological properties and in indigenous healthcare uses. The three well documented bio active compounds from *Azadirachtaindica* are A)Azadirachtin B) Nimbin C) Nimbolin – B.The three bio active compounds from neem were docked with chitin synthase in the grid box generated based on the active site information given by castp3.0 server (Fig. 6)

Azadirachtin when docked with chitin synthase in PYRX showed interaction with amino acidsASP 869, TYR 560, PRO 1015, LYS 1151, ASP 1016, SER 1017 with a docking score of -7.8 k cal/mol. Nimbin showed interaction with GLN 830, PRO 961, SER 968, VAL 579, VAL 761, ALA 700, ASN 970, ARG 767. With a docking score of -7.1 kcal/mol and Nimbolin B shows interaction with LEU 1067, ILE 917, LEU 921, VAL 516, ARG 519, PRO 1102 with a binding score of -8.4 kcal/mol

Among the three bio active compounds of *Azadirachtaindica*nimbolin-b showed strong binding affinity to chitin synthase in the insilico docking studies(Table. 2).

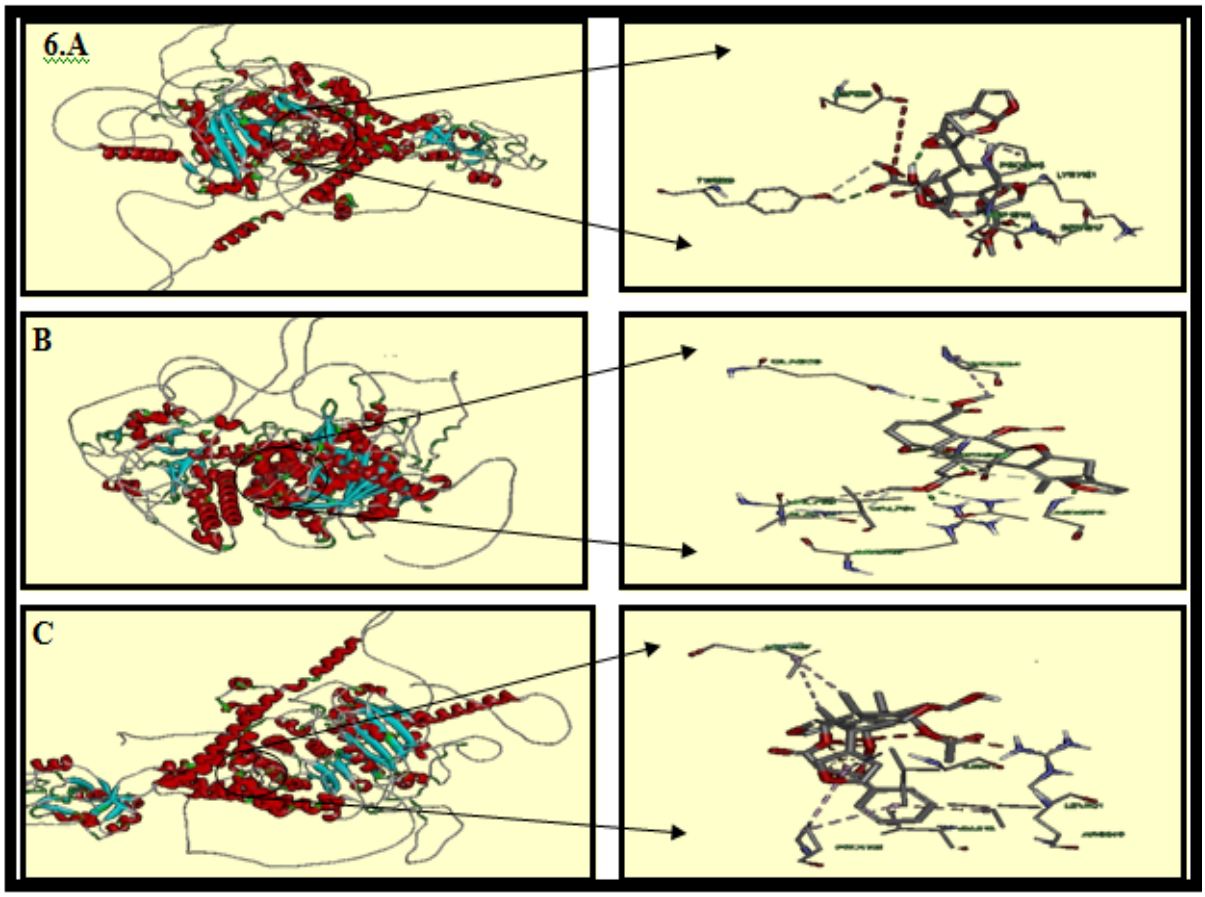

Fig. 6 Interaction of Bioactive compounds from *AzadirachtaIndica*- 3 A) Azadirectin, B) Nimbin and C)Nimboline –B with Chitin Synthase

S.No	Enzymes/Bio Compound	Interacting amino acids / Pocket amino acids	Docking Score

			kcal/mol
01	Azadirachtin	ASP 869, TYR 560, PRO 1015, LYS 1151, ASP 1016, SER 1017	-7.8
02	Nimbin	GLN 830, PRO 961, SER 968, VAL 579, VAL 761, ALA 700, ASN 970, ARG 767.	-7.1
03	**Nimboline –B**	**LEU 1067, ILE 917, LEU 921, VAL 516, ARG 519, PRO 1102**	**-8.4**

Tab: 2 *Azadirachta Indica* interacting amino acids and molecular docking score

Molecular docking of Curcumin a bioactive compound from *Curcuma longa with*chitin synthase showed specific n interaction.

Curcumin has low stability and low bioavailability, it has been proved that this compound induced apoptosis signalling and is also known to block cell proliferation pathway. Among its many uses this compound may also be an effective bio pesticide to increase the resistance to diseases in plants and crop fields.

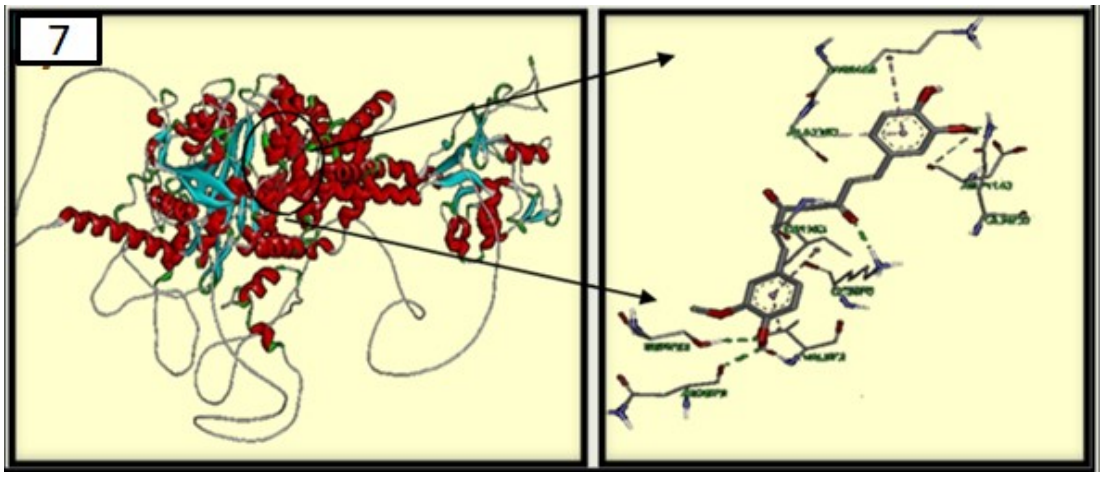

Fig.7 Interaction of Bioactive compound of *Curcuma longa*,Curcumin with Chitin Synthase.

S.No	Enzymes/Bio Compound	Interacting amino acids / Pocket amino acids	Docking Score kcal/mol
01	Curcumin	LYS 1159, ALA 1160, ASP 1142, GLN 233, ILE 1162, LYS 978, SER 763, VAL 972, ASN 970	-7.4

Table: 3 molecular interactions and docking score of Curcumin

Curcumin showed potential hydrogen bonds with the active site amino acid residues (Fig. 7). It formed interactions with Chitin Synthase with the help of interacting Atoms or pocket atoms. Curcumin - LYS 1159, ALA 1160, ASP 1142, GLN 233, ILE 1162, LYS 978, SER 763, VAL 972, ASN970with the binging score -7.4 kcal/mols given in the table 3.

Conclusion

The docking studies derived in this research revealed some potential compounds and paved a route for structure-based fungicide discovery. Our results on binding interactions between chitin synthase and bioactive compounds derived from plants would be helpful for formulating specific natural concoctions against *Magnaporthe oryzae*. This study gives an insight into potential interaction and binding affinities of 9bio compounds Anonaine, Asimilobine, Azadirachtin, Corypalmine, Curcumin, Nimbin,Nimbolin-B,Nornuciferine and Reticuline. Anonaine and Nimboline-Bhave the highest dock score value

among the selected ligands.The docking output revealed good binding affinity values ranging from –7.0 to –8.5kcal/mol which confirms their resilience potency. Furthermore, ligands Anonaineand Nimbolin-B scored higher binding affinity values of −8.4 and –8.5kcal/mol respectively.

All the selected compounds possessed good in isilico properties, demonstrating their use in further development into active antifungal compounds, however Anonaine and Niomboline –B showed highest binding affinity, further molecular dynamic simulation and fine tuning of the molecule may result in effective fungicide against *Magnaporthe oryzae*. Also, experimental characterization is needed for further validation of the protein target. Further research in this direction will aid in sustainable management of fungal pathogens which cause substantial yield loss in crop plants.

The research outcomes set a course for structure-based drug design and revealed some potential molecules for future molecular dynamic simulationsto create refined potential anti-fungal molecules.

Natural concoction containing leaf extracts of *Annona squamosa* and *Azadirachta indica* are recommended against *Magnaporthe* oryzae based on our preliminary insilico studies. Brahmastra containing leaf extracts of neem and custard apple is best suitable for spraying against the fungal pathogen *Magnaporthe oryzae.*

Acknowledgements: The Authors acknowledges financial support from Dr. B. R. Ambedkar University, Srikakulam and technical support from Prof A. Dinakararao's Lab, Department of Bioinformatics, Pondicherry Central University. Authors claim no conflict of Interests.

Conflicts of interest: Authors declared No conflict of interest.

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