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In silico analysis of chitin deacetylase and scrutinizing the fungicidal potential of colchicine

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Abstract
Melons, were oppressively afflicted by infection of powdery mildew. *P. xanthii* is a major causative agent of powder mildew in melons. Chitin deacetylase is a potential target for development of new fungicides to control the *P. xanthii* infection, since it is involved in the evading of pathogen from plant immunity. Chemical fungicides were paramount to control powdery mildew in melons where these fungi develop the resistance to the available fungicides. Hence there is an urgent need to develop newer fungicides to control the infection of *P. xanthii*. In our study, the domains localized in CDA were identified and interaction of colchicine to catalytic active domains was studied using molecular interaction studies. This study revealed the binding of colchicine to NodB domain a catalytic domain of CDA with least binding energy. Further, the stability of CDA-colchicine complex was assessed using normal mode analysis, which confirmed the high stability of complex. Based on the molecular interaction and stability analysis it was evident that colchicine can used for the development of potential fungicides to control powdery mildew in cucurbits.

Keywords: Colchicine, cucurbits, fungicide, *P. xanthii*, and chitin deacetylase

Introduction
Cucurbits are economically important plants with edible fruits (Cui *et al.*, 2022) [4]. Cucurbits were commonly known as melons which belongs to the family of Cucurbitaceae consists of 120 genera and more than 800 species (Ajuru and Nmom, 2017) [1]. Cucurbits species are cultivated as a cash crop for human consumption. In addition to edible property, cucurbits also contain the medicinal values like digestive stimulant, lactogogue, antiseptic, analgesic, and antiemetic (Ajuru and Nmom, 2017) [1]. Melons were cultivated in different climatic conditions and monsoon weather which favours for pest and pathogen attack (Cui *et al.*, 2022) [4].

Powdery mildew is a serious pathogen infecting cucurbits, the disease caused by a fungal pathogen. The major fungal pathogens causing powdery mildew are *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Rhouma *et al.*, 2022) [15] which reduce the yield drastically and affects quality of edible melons (Saharan *et al.*, 2019) [16] and the yield reduction will be up to 50-70 per cent because of powdery mildew infection (Pitchaimuthu *et al.*, 2012) [13]. The melons are grown for different climate conditions in every season, hot to humid condition is an optimal climatic condition for fungal attack, especially for *P. xanthii* attack (Cui *et al.*, 2022; Rhouma *et al.*, 2022) [4, 15]. There are 21 identified races of *P. xanthii* throughout the world and there is continuous formation of new races (Cui *et al.*, 2022) [4]. Fungicide application is an immediate control measure for *P. xanthii*. Several reports documented the resistance of *P. xanthii* to fungicides (McGrath and Shishkoff, 2003; Cui *et al.*, 2022) [11, 4], which necessitates the development of new agrochemicals for controlling *P. xanthii*.

Chitin deacetylase a novel target for controlling the multiplication fungus *P. xanthii* in host. This enzyme involved in formation of chitosan from cell wall chitin by hydrolysing N-Acetylglucosamine, N-acetamido group (Martínez-Cruz *et al.*, 2021) [10]. De acetylation of chitin makes plants not to recognizes the fungal attack and helps to escape from plant immunity.

Alkaloids, extracted from the plant act as antifungal agents (Yoon *et al.*, 2013) [18]. Colchicine ($C_{22}H_{25}NO_6$), an amino alkaloid synthesised in different tissues of *G. superba*, possess several medicinal properties, and used for crop improvement programmes. Crude extract of *G. superba* exhibited potential antifungal activity against *Candida albicans* (Khan *et al.*, 2008) [5]. Chemical fungicides are predominant till date for controlling *P. xanthii*, which are hazardous to plant and animals and causes environmental deterioration.

To minimize the effect of synthetic fungicides, new green fungicide has to be developed. Considering the above-mentioned problems, the study was conducted to develop a green agro-chemical for controlling *P. xanthii* attack on cucurbits. Colchicine an alkaloid with incredible anti-microbial activity was also studied for interaction with chitin deacetylase (target protein of *P. xanthii*). We studied the presence of sheets, turns, helices and domains in chitin deacetylase protein where colchicine interacting with target protein and we developed the 3D structure of chitin deacetylase using *in silico* tools. For the first time, we are reporting the that colchicine could be used in developing fungicides to supress the virulence of *P. xanthii*.

Materials and Methods

Retrieval of plant fungal protein sequence

The protein chitin deacetylase was selected through an antifungal target protein for fungicide designing (Martínez-Cruz *et al.*, 2021) [10]. Protein sequence of chitin deacetylase (Uniprot Id: A0A6S4IFB1) from *P. xanthii* was retrieved from Uniprot database (accessed on August, 2022).

Structural analysis of Chitin deacetylase

Secondary structure prediction

Presence of helix, turns and sheets in protein were analysed using the CFSSP (Chou and Fas man secondary structure prediction) (http://www.biogem.org/tool/chou-fasman/) (Kumar, 2013) [7] and PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/) (McGuffin *et al.*, 2000) [12]. This disclosed the percentage of secondary structural elements in the protein. In addition to sheets, helix and turns domains localized in the protein were also predicted using Inter pro scan (https://www.ebi.ac.uk/interpro/search/sequence/) (Quevillon *et al*., 2005) [14].

Tertiary structure prediction

Since, no availability of 3D structure of protein, the obtained protein sequence was modelled using the Ros TTA Fold modelling method available in Robetta webserver (https://robetta.bakerlab.org/) (Kim *et al.*, 2004) [6]. Rose TTA fold predicts the protein structure using the artificial intelligence and three tract neural network model, further quality of the modelled protein structure was assessed using the SAVES server (https://saves.mbi.ucla.edu/). ERRAT and PROCHECK programs available in SAVES server was used to critically assess the protein structure.

Ligand Preparation

The 2D conformer of colchicine and Mancozeb was retrieved from the Pub Chem (Pub Chem ID: 6167) and (Pub Chem ID: 13307026) database downloaded in the SDF format. Further, 2D conformer (SDF) converted into 3D structure (PDB) using Open Babel software available in PyRx V0.8.

Protein preparation

The 3D structure of Chitin deacetylases modelled using Rose TTA Fold was employed for interaction studies. Protein was prepared with the help of PyRx software, then macromolecule was converted into pdbqt (Protein Data Bank, Partial Charge (Q), & Atom Type (T)) format.

Active site prediction

Catalytic active sites were predicted It predicted the active site residues based on computational geometry using the using the CASTp server (Computer atlas for Surface Topography of Proteins - http://sts.bioe.uic.edu/castp/calculation.html) (Tian *et al.*, 2018) [17]. The model built using Rose TTA Fold was used for active site prediction.

Molecular Docking and Interaction studies

Molecular interaction studies were carried out to find the anti-fungal activity of colchicine against the chitin deacetylase (Uniprot Id: A0A6S4IFB1) of *Podosphaera xanthii.* In virtual screening, commercial fungicide triflumizole was used as internal control and colchicine used as novel phytochemical for fungicidal activity. The physio-chemical properties of the internal control and colchicine was given in Table 1. The ligand energy, was minimised using the Unified Force Field (UFF) for 200 steps. Grid box was created by covering the active sites disclosed in literature survey and by CASTp server. The coordinates of the grid box used in the study was X = 10.712, Y = 14.988 and Z = 13.232. Virtual screening was carried out by employing Auto Dock Vina module in PyRx. During the process of docking, ligands were made as flexible molecule to generate confirmations with the value of 8 exhaustiveness. The docked complex of macromolecule and ligand was visualized using the BIOVIA Discovery Studio Client 2021 (https://www.3ds.com/products-services/bio via/). Further, compactness of interaction was verified using molecular dynamics analysis.

Macromolecule and ligand stability analysis

Normal mode of protein-ligand complex was calculated using normal mode analysis of internal coordinates by pooling the motion of protein with the use of iMODS server (https://imods.iqfr.csic.es/) (López-Blanco *et al*., 2014) [9]. It calculated the deformability, B-factor, and Eigen values. Hinges in atom residues defines probability of deformability of main-chain. The Eigen values were determined based on motion stiffness. Eigen value is directly proportional to the energy required to break the complex, higher Eigen value favours higher stability of complex and lower Eigen value confers lower stability of complex. This server performs the dynamics in rapid manner than the classic molecular dynamics.

Results and Discussion

P. xanthii, the causative fungus of the disease, powdery mildew to cucurbits, causes dramatic yield loss. Further, the fungus develops resistance to available fungicides and fungicides are hazardous to the environment, which urges the development of newer fungicides. The current *in silico* study identified the colchicine as a novel green fungicide which might be used in development of novel green fungicide in controlling the disease caused by *P. Xanthii*

Secondary structure prediction

Sequence secondary structural analysis revealed the presence of 45.7 per cent alpha helix, 40.1 per cent beta sheets and 12.0 per cent beta turns. The presence of helix, sheets and turns identified in secondary structure prediction analysis with position were given in the figure 1A and 1B. The higher percentage of helix and sheets in protein suggested the thermos table nature of the chitin deacetylase. Banik *et al.* (2022) [2], predicted a similar the higher percentage of helix and sheets in poly protein of fungus *Rhizopus oryzae.* The

chitin binding domain and NodB domains were identified in CDA of *P. xanthii* (Table 2).

Tertiary structure prediction

Rose TTA Fold modelling method followed for tertiary structure prediction of macromolecule, resulted in model with 0.79 confidence. Further, validation of predicted model using ERRAT and PROCHECK module of SAVES server, resulted in 96.97 per cent overall quality score for the model and Ramachandran plot showed the high compactness of modelled 3D structure of CDA. In RC plot, all the amino acids were in the allowed region, except glycine and proline (Figure 2).

Active site prediction

Active site identification using CASTp server deduced the presence of 53 active site pockets, and the selected finest pocket was the first active site pocket which has an Area (SA) $Å^2$ and Volume (SA) $Å^3$ of 686.911 and 1600.584 respectively, followed by second finest active site pocket with 119.75 (Area) and 54.304 (Volume).

Molecular interaction studies

CDA is directly involved in the pathogenesis for cucurbits. Interaction of internal control (triflumizole) and Colchicine (novel phytochemical) was analysed using the molecular docking. Triflumizole showed the interaction through hydrogen bond against CDA with binding energy of -6.4 Kcal / mol to TRY207 (Figure 3). Colchicine interacted with CDA by two hydrogen bonds with binding energy of -7.1, hydrogen bond forms between the oxygen atom of colchicine with TYR207 of CDA and hydrogen atom of colchicine with HIS271 of CDA (Figure 3). Colchicine bound and interacted to CDA with lowest binding energy while comparing with available commercial fungicides, which conveyd that colchicine could be used as a novel fungicide (Table 3). Khan *et al.* (2008) also confirmed that *G. superba* extract could inhibit the fungal growth of *C. albicans*. Colchicine was binding to the catalytic active domain NodB which was involved in de acetylation of chitin (Liu *et al.*, 2019) [8].

Normal mode analysis

Normal mode analysis using the iMODS server calculated the vibrational modes (internal coordinate's dynamics) and protein flexibility for CDA-Colchicine complex. The deformability values resulted in range of 0-1, more residues with the deformability with range of less than 0.5 (Figure 4A). Lower deformability infers lower hinges in CDA-Colchicine complex. B-factor value of complex reveals the equivalent of NMA to RMS (Figure 4B). Possibility for complex deformation was calculated using eigen value by assessing the stiffness of Cα motion, which resulted in 1.479 x 10^{-5} for CDA-colchicine complex (Figure 4C). The higher eigen value denotes the higher stability of CDA-colchicine complex. Covariance matrix represents the connection between the motion of residues, red colour denotes the positively correlated, white defines the uncorrelated between residues and blue colour represents the negatively correlated between residues (Figure 4D). The higher stability of complex deduces interaction between colchicine and CDA was stronger and may colchicine can inhibit the CDA. Normal mode analysis was used by Choudhury *et al.* (2021) to estimate the dynamics of protein and ligand complex, where they reported the similar eigen value 2.17 x 10^{-5} for the higher stability of ligand with protein.

Table 1: Chemical and physical properties of Colchicine and internal control

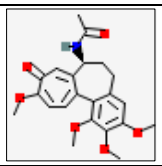
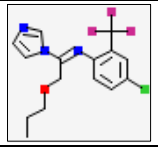
Ligand	Molecular weight	Molecular Formula	Rotatable bond	Structure
Colchicine	399.4	$C_{22}H_{25}NO_6$	5	
Triflumizole (Internal control)	345.75	$C_{15}H_{15}ClF_3N_3O$	6	

Table 2: Domains present in chitin deacetylase of *P. xanthii*

Protein	Domain	Region
Chitin deacetylase	Chitin binding	23-67
	NodB	101-297

Table 3: Interaction of internal control and colchicine with CDA

Ligand	Binding Site	No. of H-Bond	Bond length	Binding energy
Colchicine	TYR207, HIS271	2	2.05, 2.27	-7.1
Triflumizole (Internal control)	TYR207	1	2.38	-6.4

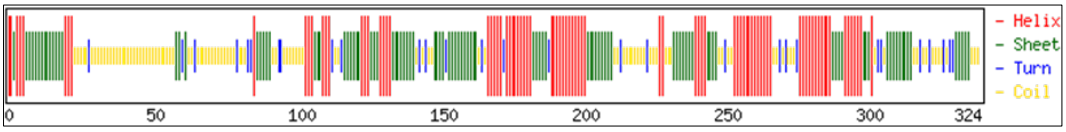


Fig 1A: Helix, turns and sheets in chitin deacetylase of *P. xanthii*

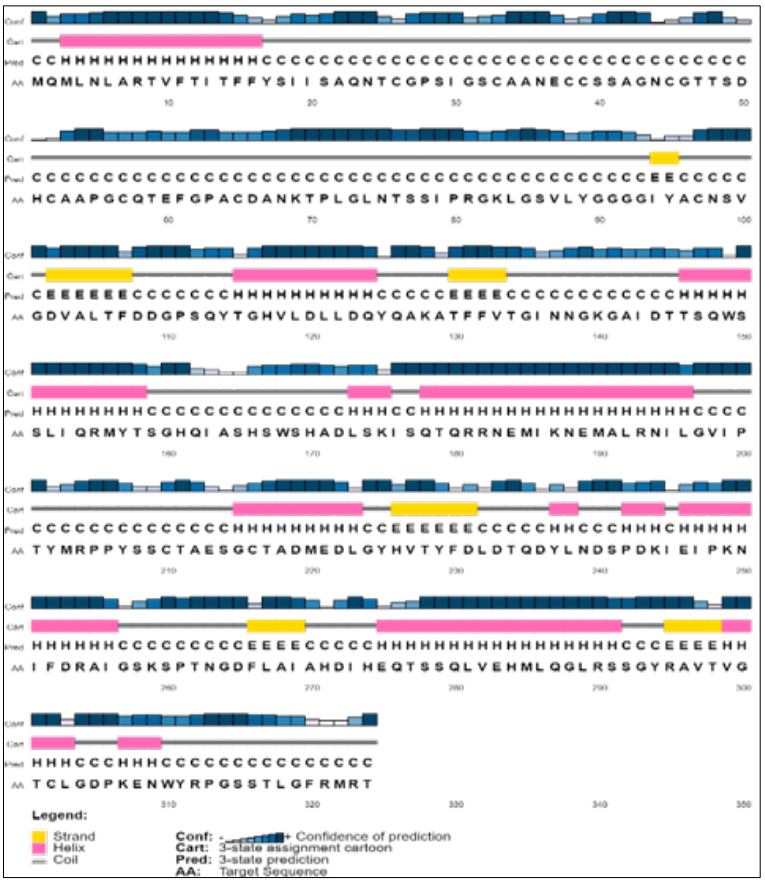


Fig 1B: Confidence of identified secondary structure elements

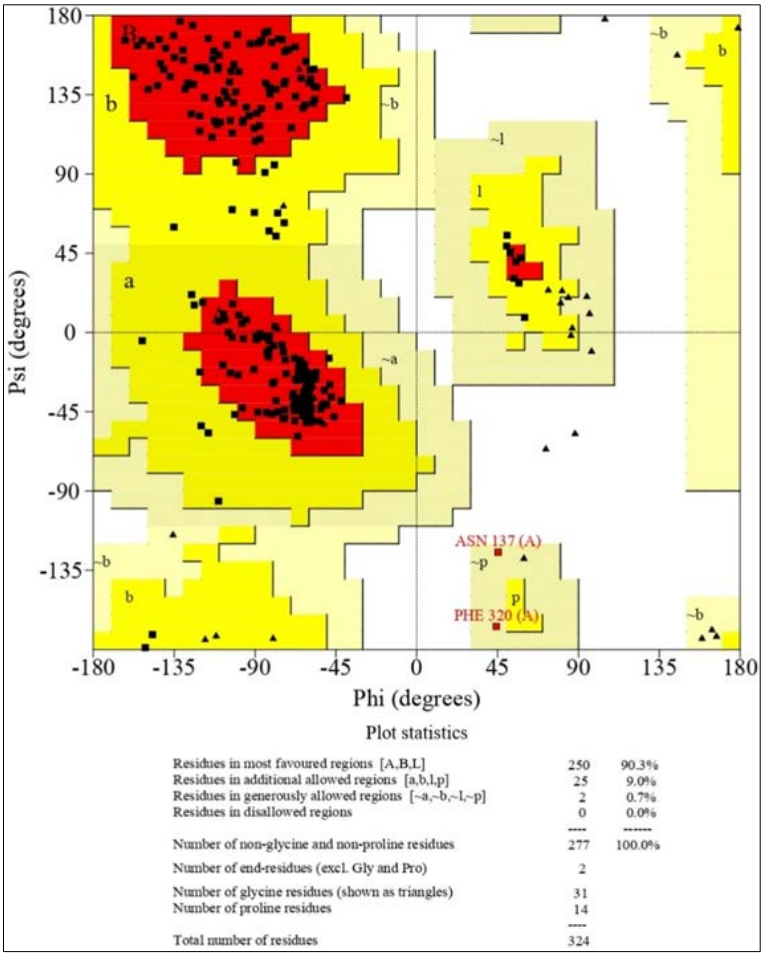


Fig 2: Ramachandran plot depicting the quality of CDA 3D structure

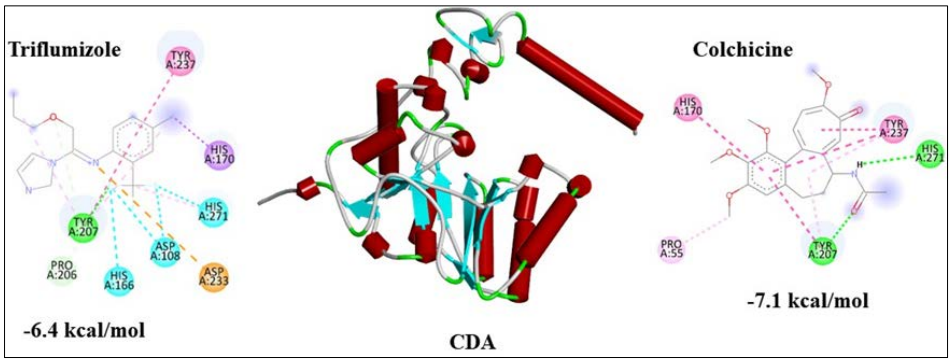


Fig 3: Hydrogen bond formed between active sites and ligands

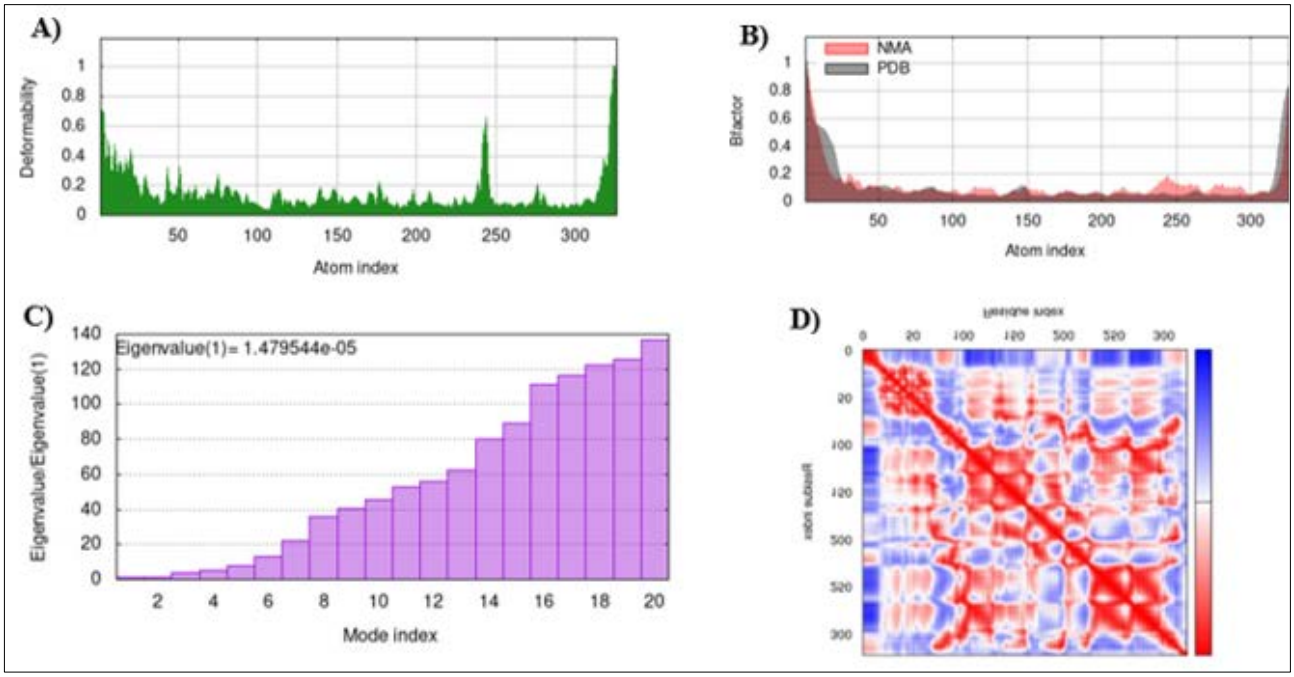


Fig 4: Dynamics studies using Normal Mode analysis (A) Deformability, (B) B-factor, (C) eigen value and (D) covariance

Conclusion

Colchicine interacted with CDA using least binding energy than available control triflumizole. Further, this could be used for the controlling the infection causes by *P. xanthii*to cucurbits on a routine way as colchicine was estimated to accumulate at higher concentration in Gloriosa than any other plant species. Based on the results obtained, it is suggested to validate the efficiency colchicine to arrest the fungus growth through *in vivo* studies, and this can be assessed for broad spectrum antifungal activity. The modelled structure of *P. xanthii* CDA from the study could be used for screening other antifungal agents.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors Contribution

S. Selva Babu, R. Gnanam, M. Jayakanthan, N. Saranya N. Senthil and J. Suresh conceived the ideas and planned the experiments. Selva Babu S performed the analysis. R. Gnanam, M. Jayakanthan and N. Saranya supervised *in silico* analysis. R. Gnanam oversaw the overall experimental procedure and supervised the findings, and structured the complete manuscript. R. Gnanam, N. Senthil and J. Suresh provided academic support for analysis. All authors provided substantial feedback, discussed the results, and contributed to the final manuscript.

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