Mysore J. Agric. Sci., 58 (2) : 274-284 (2024)

Computational Profiling of Potential Endophyte Metabolites against Tobacco Mosaic Virus : A Strategy for Antiviral Drug Discovery

Shadab M. Khatib[1], M. Bharath[2], P. S. Pooja[3], Kopparthi Amrutha Valli Sindhura[4], Mantesh Muttappagol[5], Anil Annappa Gudimani[6], K. S. Uday Durga Prasad[7] and C. N. Lakshminarayana Reddy[8]

[1,2,3,5,6,7&8]Department of Plant Pathology and [4]Department of Agricultural Entomology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

e-Mail : cnlreddy@gmail.com

Authors Contribution

Shadab M. Khatib : Conceptulization, investigation, data analysis and manuscript preparation;

M. Bharath, P. S. Pooja, Kopparthi Amrutha Valli Sindhura & Mantesh Muttappagol : Conceptulization and Methodology;

Anil Annappa Gudimani & K. S. Uday Durga Prasad : Literature mining;

C. N. Lakshminarayana Reddy : Conceptulization, supervision and editing

Corresponding Author :
C. N. Lakshminarayana Reddy

Received : December 2023
Accepted : January 2024

Abstract

Plant viral diseases pose a significant threat to global agriculture, jeopardizing sustainability and productivity. Among these, tobacco mosaic virus (TMV) is considered as a devastating virus and is still out of control due to the lack of availability of efficient functional antagonist chemical molecule for its management. In recent years, molecular docking has emerged as an important tool for drug discovery and design, offering the means to predict the nature of binding interactions between ligands and target proteins. To explore the antiviral activity of potential fungal endophyte, CSR1 *(Macrophomina pseudophaseolina)* against coat protein (CP) of TMV, current study was conducted to know the interaction of endophyte metabolites with TMV CP through *in-silico* docking analysis. The protein structures of virus pathogenicity determinants such as CP, was predicted with the aid of *ab-initio* modelling. The metabolite library of CSR1 endophyte which was available was used for docking studies. *In-silico* prediction of interaction between endophyte metabolites and CP were performed. The molecular docking analysis showed that the 22 promising metabolites from the endophytes belonging to different groups have exhibited better binding affinity with TMV CP with favorable binding energy ranging from -6.02 to -7.30 kcal/mol. These findings offer preliminary evidence of the potential anti-TMV activity of compounds sourced from the CSR1 endophyte, indicating their promise in combatting this viral pathogen.

Keywords : *In-silico* analysis, Binding affinity, Antiviral compounds, Drug discovery, Protein-ligand interaction

Plant diseases caused by viruses have become a major concern in the agriculture production leading to the substantial economic loss and jeopardizing agricultural development across the world (Jones, 2021). Among the most destructive plant viruses, Tobacco Mosaic Virus (TMV) stands out as a major threat. TMV is a positive-sense single-stranded RNA virus belongs to the genus, *Tobamo virus.* It infects plants belonging to the *Solanaceae* family including tobacco, potato, tomato, pepper and various others crops resulting in an enormous agricultural losses (Wang *et al*., 2020 and Xia *et al.*, 2019). The genome of TMV comprises four distinct open reading frames (ORFs) each playing a crucial role in the multiplication of TMV particles, its ability to infect and spread within host plants. The 5' proximal of first two ORFs encode readthrough proteins with molecular weights of 126 and 183 kDa containing methyltransferase (MET), helicase (HEL) and polymerase (POL) domains which

are involved in genome methylation, replication, and transcription process. The ORF 3 encodes the 30 kDa movement protein (MP), which facilitates the intercellular movement of the virus within the plant and ORF 4 encodes the 17 kDa coat protein (CP), essential for the assembly of virus particles and protection of the viral RNA (Ibrahim *et al*., 2019).

In light of the challenges posed by plant viral diseases and the limitations of conventional control methods, the concept of utilizing endophytes as biocontrol agents gaining prominence. Therefore, the use of endophytes as biocontrol agents is drawing special attention as an efficient measure for management of plant viral diseases. Endophytes are the storehouse of many bioactive compounds or metabolites with antiviral properties and positioning endophytes as promising candidates for the development of sustainable approaches to combat plant pathogens (Gunatilaka, 2006). In the recent decade, computational biology has emerged as a powerful tool driving numerous breakthroughs in the field of virology (Pappas *et al*., 2021). Concurrently, drug development from endophytes has been demonstrated against spectrum of debilitating diseases including viral infections (Linnakoski *et al.*, 2018 and Peters *et al*., 2020). Moreover, studies have lent credence to exploration of secondary metabolites from both plants and endophytes as lead compounds against druggable targets of diseases using computational approaches (Shode *et al*., 2021 and Li *et al*., 2022).

With this backdrop of innovation and potential, the present study employs computational approaches to screen fungal endophyte metabolites interactions with the TMV CP (Tobacco mosaic virus coat protein) for the development of antiviral agents. The study outcome provides new insights into the development of eco-friendly and effective strategies for managing plant viral diseases and safeguarding agricultural productivity.

Material and Methods

Endophyte Collection and Extraction of Secondary Metabolites

The potential endophyte isolate CSR1 identified as *Macrophomina pseudophaseolina* isolated from the roots of *Cassia tora* which was previously characterized during the year, 2021 by Nandan was collected from the Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru. It is important to note that the choice of endophyte was made based on its previous characterization and its potential significance in the management of plant diseases. The primary objective of this study was to explore the production of antimicrobial diffusible compounds by the fungal endophyte CSR1. To achieve this, the extraction of the secondary metabolites produced by the endophyte CSR1 was carried out through the ethyl acetate extraction method as described by Pansanit and Pripdeevech (2018) with slight modifications as mentioned below.

The 10 mm mycelial disc of actively growing CSR1 endophyte on potato dextrose agar (PDA) media was inoculated to a 1000 mL conical flask containing 200 mL of PDB and incubated at 27 ± 1°C. After 20 days of incubation, the grown mycelial mat was removed using sterile forceps and the filtrate was filtered through Whatman No. 1 filter paper. The 200 mL filtrate was mixed with an equal volume (200 mL) of ethyl acetate (1:1 v/v ratio) and subsequently, the mixture was partitioned in a 500 mL separating funnel. In the separating funnel, a lower fraction containing the broth was discarded and the upper solvent fraction containing the metabolites was collected into a round bottom flask. The collected metabolites in the solvent fraction were subjected to a rotary evaporation at 40°C to evaporate ethyl acetate. Further, to get concentrated extract of secondary metabolites, it was subjected to a vacuum rotary evaporator with reduced pressure at 40°C. Finally, the concentrated ethyl acetate extract of fungal endophytes was subjected to LC-MS/MS analysis.

Characterization of Secondary Metabolites from CSR1 Endophyte through Quadrupole Time of Flight Liquid Chromatography Mass Spectrometry (Q-TOP LC-MS/MS) Analysis

LC-MS/MS is a simple, robust interface which can be applied to decipher a wide range of biological molecules. The ethyl acetate extracts of fungal isolate

CSR1 was subjected to Agilent Q-TOP LC-MS/MS facility available at Indian Institute of Science (IISc), Bengaluru. In MS, the molecule is converted to an ionized state and is detected based on its mass to charge ratio (Pitt, 2009). LC-MS/MS method has two polarity modes, a negative and a positive mode. The difference is that, positive ion mode charges through protonation while negative ion mode charges the analyte through deprotonation. The LC-MS analysis with positive mode ion is generally preferred as more compounds are expected to ionize in this mode (Liigand *et al*., 2017). Hence, fungal secondary metabolite characterization was done in positive mode ion as described by Tang *et al*. (2020).

Chromatographic separation of molecules was carried by Agilent poroshell 120 (4.6 × 150 mm) column. Lyophilized CSR1 endophyte extract was dissolved in 0.1 per cent formic acid (solvent A) and acetonitrile with 0.1 per cent formic acid (solvent B) and a volume of 5 µL was injected into the mobile phase. The metabolite peaks obtained in the chromatogram were analyzed through Bruker Compass Data analysis software.

To decipher the chromatogram peaks, the raw data was initially converted to mzXML format with the aid of MS Convert and subjected to MZmine 3 software (Pluskal *et al*., 2010). It is an open-source framework for visualization, processing and identification of metabolome profile data. Identification of processed chromatogram peaks was performed by connecting to online database search tools such as PubChem (Kim *et al*., 2016), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) and Chemical Entities of Biological Interest (ChEBI) (Degtyarenko *et al*., 2007) directly from MZmine3 interface. Further, the detected CSR1 endophytic secondary metabolites were subjected to *in-silico* docking analysis to find the binding affinity of these metabolites with viral protein.

Ligand Source and Preparation

The compounds identified through LC-MS/MS analysis were selected for molecular docking studies and three-dimensional (3D) conformers of these ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) database in SDF and MOL formats. As a positive control, ningnanmycin, a well-established antiviral agent against TMV infection was chosen (Zhao *et al*., 2015). The retrieved 3D structures of ligands were converted into single SDF file using Open Babel and single SDF file containing multiple ligand compounds were optimized and further, converted to PDB format using PyRx software (Hanwell *et al*., 2012). This conversion to PDB format was crucial for compatibility with the subsequent molecular docking analysis. To streamline the ligand preparation and docking process, the optimized ligands with the lowest energy were loaded to AutoDock-MGL Tools (Morris *et al*., 2009), the Gasteiger charges were added and standard processes were used to obtain the PDBQT files, which are essential for setting up and conducting molecular docking studies with the TMV. This comprehensive approach allowed for the assessment of potential antiviral activity in comparison with the positive control, ningnanmycin.

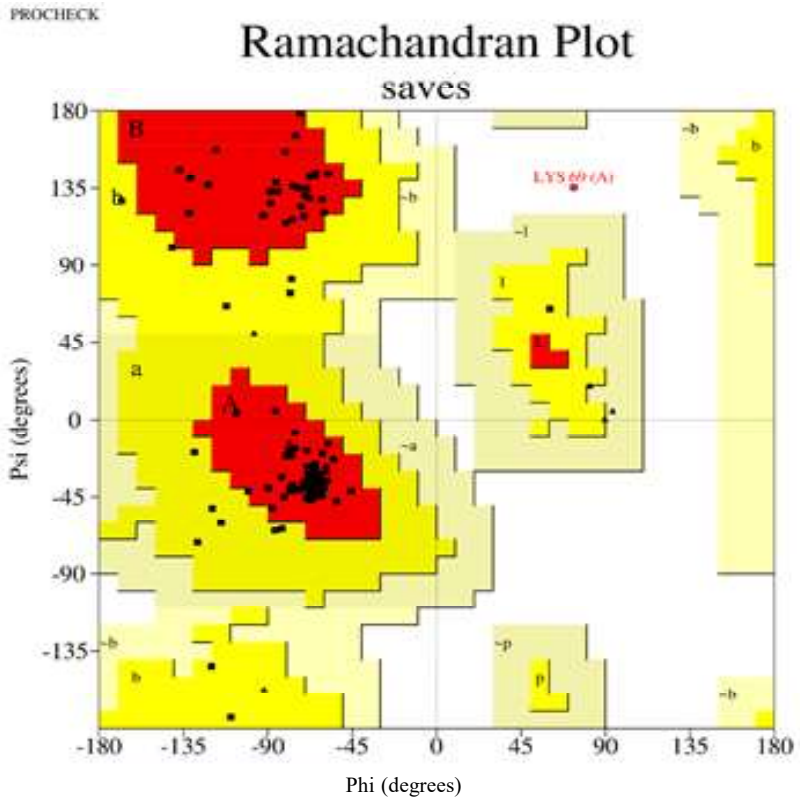
Source of Viral Target Proteins

The TMV CP was selected as a receptor protein for the subsequent docking studies. The amino acid sequences of the selected protein were downloaded from NCBI Database with accession ID: (L35074.1) (Likhith & Peter, 2023 and Hiremath *et al*., 2021). Since, there was no significant sequence similarity found with the target receptor protein, the protein structure for TMV CP was built *ab-initio* through Iterative Threading Assembly Refinement (I-TASSER) (https://zhanglab.ccmb.med.umich.edu). The quality of the predicted structure was analyzed using structural validation algorithm SAVES v6.0. Additionally, to validate the top-performing 3D model, a comprehensive evaluation was conducted through a Ramachandran plot analysis using the PROCHECK server (Laskowski *et al*., 1993).

Preparation of Target Sites and Active Site Prediction

The downloaded 3D protein structures were checked for the presence of any improper bonds, side-chain

anomalies and missing hydrogens using PyMOL software (Delano, 2009). Subsequently, to streamline the subsequent docking analysis, all the water molecules, complex molecules, ions and ligands of the proteins were removed in Biovia Discovery Studio 2020 (Biovia, 2020). The optimized PDB structures were uploaded to AutoDock-MGL Tool (Morris *et al*. 2009), succinctly, polar hydrogens were added and standard processes were used to obtain the PDBQT files. The active sites of the proteins were determined using the Computed Atlas for Surface Topography of Proteins (CASTp) (http:// sts.bioe.uic.edu/castp /index .html?2011) and Biovia Discovery Studio 2020.



Plot ststistics

Residues in most favoured regions (A,B,L)	123	88.5%
Residues in additional allowed regions (a,b,l,p)	15	10.8%
Residues in genemusly allowed regions (~a,~b,~l,~p)	0	0.0%
Residues in disallowed regions	1	0.7%
Number of non-glycine and non-proline residues	139	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine and residues (shown as triangles)	6	
Number of proline residues	8	
Total number of Residues	155	

Based on an analysis of118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Fig. 1 : Ramachandran plot analysis for TMV CP (Tobacco mosaic virus coat protein) predicted with the aid of I-TASSER. Plot was generated by uploading protein in PDB format to PROCHECK program of SAVES 6.0 tool. Area of the Ramachandran plot is shaded according to various degrees of energetic stability: most favoured region (red), additional allowed region (dark yellow), generously allowed region (light yellow) and disallowed region (white)

Molecular Docking

The optimized ligands and proteins in PDBQT format were subjected to molecular docking with the aid of AutoDock Vina software (Trott and Olson, 2010). AutoDock Vina is a powerful tool for predicting the interaction between protein and ligand, by utilizing its scoring function (binding affinity). During the molecular docking process, active sites were predicted for TMV CP and the cubic grid box was set to 40.91 Å × 30.04 Å × -41.89 Å centered at 126, 126, 110 (XYZ coordinates) using the grid box generation platform of the AutoDock-MGL tools, large enough to allow for the free rotation of the ligand molecules around the selected binding site residues (Natesh *et al*., 2021). The ligand binding pose showing highest binding affinity and least root mean square deviation (RMSD) was selected, indicating a strong interaction with the protein. Protein-ligand interactions were further visualized in both 3D and 2D structures using PyMOL (Delano, 2009) and LigPlot + (Laskowski and Swindells, 2011), which provides a comprehensive understanding of how the ligands bind to the TMV CP and the specific molecular interactions at play.

Results and Discussion

The secondary metabolites from the endophyte CSR1 grown in potato dextrose broth (PBD) were extracted using ethyl acetate. The extracts of fungal isolate CSR1 were subjected to Agilent Q-TOF LC-MS/MS analysis, where the molecule is converted into an ionized state and are detected based on their mass to charge ratio (Pitt, 2009). Raw data was analyzed with the aid of MZmine 3 revealed a substantial dataset with 3960 chromatogram peaks identified within the mass-to-charge of 44.87-1805.06 m/z (mass to charge ratio). This extensive dataset was pivotal in the identification of putative 541 biologically active compounds with the aid of online database search tools such as PubChem, KEGG and ChEBI directly from MZmine3 interface. In this study we retrieved 289 3D structures of ligands from the PubChem database, which were obtained in both SDF and MOL formats. These ligands were energy minimized and subsequently converted to PDB format.

***Ab-initio* Protein Modelling, Active Site Prediction and Refinement**

Due to the unavailability of the template structure for TMV CP in Protein Data Bank (PDB), the three-dimensional (3D) conformer was built with the aid of I-TASSER. Among the top five models predicted for CP, we selected the model with a high confident score (C-score) of 1.05 as the best model. The estimated TM score and root mean square deviation (RMSD) of the selected model were 0.86 ± 0.07 and 2.8 ± 2.0Å, respectively. The TM score serves as a key indicator of topological similarity between protein structures and ranges from zero to one, with higher values indicating greater structural resemblance (Zhang and Skolnick, 2005). These findings are significant as they provide insights

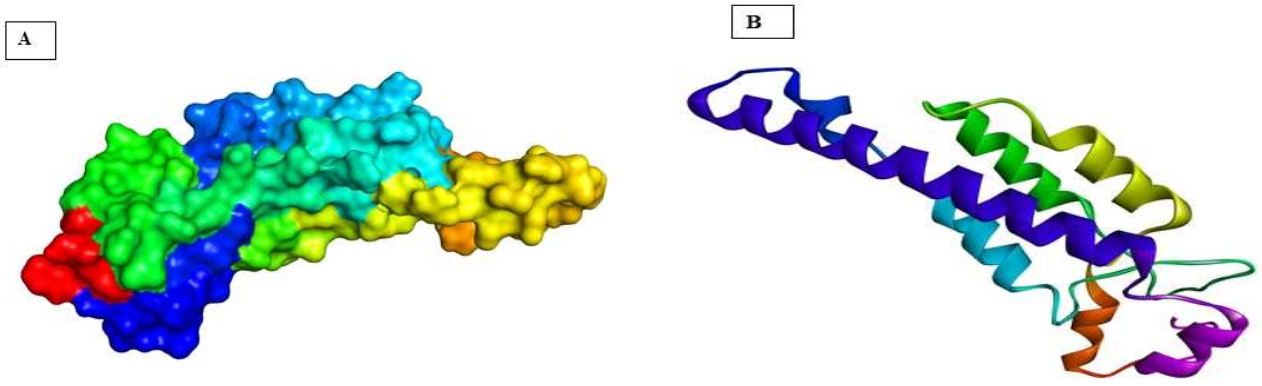


Fig. 2 : The three (3) dimensional visulalization of TMV CP (Tobacco mosaic virus coat protein) deduced using PyMOL software. A) Surface representation B) Cartoon representation. Coiled structure represents alpha helix, parallel and anti-parallel plated arrows represent beta sheets, the thread like structures represent loops and turns

into the construction and quality assessment of the TMV CP model, which is essential for the subsequent steps in our research.

The stereochemical quality of a protein model can be assessed by using a Ramachandran plot generated by the PROCHECK program. This algorithm scrutinizes a protein's structure in a plot examining its backbone conformation, by showing the - phi (φ) and psi (ψ) - angles for each residue of a protein (Ravikumar *et al.,* 2019). In Fig. 1, the plot visually represents the distribution of amino acids within various conformations including with most favoured (88.5%), additional allowed region (10.8%), generously allowed region (0.0%) and disallowed region (0.7%). Due to its good quality with more number of amino acid residues in most favoured region (123 amino acids), the model was found to be suitable for docking analysis. For a more comprehensive understanding of the TMV CP structure, fig. 2a and 2b provide visual representations of the protein in both cartoon and surface forms. These illustrations offer valuable insights into the structural features and characteristics of the TMV CP.

In-silico Interaction of CSR1 Fungal Endophyte Metabolites with TMV CP

The TMV CP plays an important role in viral pathogenesis process such as replication, translation and both intracellular and intercellular movements. Hence, the identification of potential antiviral metabolites would be a great choice in managing the spread of virus. In our current study, we employed AutoDock Vina software to virtually screen a pool of 541 fungal metabolites against the TMV CP. This screening allowed us to assess the interactions between these metabolites and the TMV CP, while binding energies were calculated to gauge the strength of these interactions in kcal / mol (Cournia *et al*., 2017). The outcomes of this screening are critical for pinpointing which fungal metabolites show the most potential in disrupting the functioning of the TMV CP.

In molecular docking process, its common practice to use a binding affinity with -6 kcal / mol as a cut off value for determining a strong binding affinity

TABLE 1

Dock scores of top 22 metabolites (with more than -6 kcal/mol) obtained after docking analysis between TMV CP with Ningnanmycin and metabolites from fungal endophyte CSR1

Compounds	Binding affinity (kcal/mol)
Ningnanmycin (Positive Control)	-5.37
Penitrem A	-7.30
Milbemycin A4	-7.22
Cytochalasin E	-6.91
2-(1,3-Dioxo-2,3-dihydro-1h-inden-2-yl) quinoline-6,8-disulfonic acid	-6.88
(+) - Butaclamol	-6.77
Amsacrine	-6.53
Scillaren A	-6.50
Amcinonide	-6.48
3-[[3-[[2-(diaminomethylideneamino) -4-methyl-1,3-thiazole-5-carbonyl]amino] phenyl] sulfonylamino]-3-phenylpropanoate	-6.31
Tetrandrine	-6.27
Cucurbitacin D	-6.26
Mocetinostat	-6.25
Nitron	-6.19
6-fluoro-3-methyl-2-(4-phenylphenyl) quinoline-4-carboxylic acid	-6.13
2-(2-Hydroxy-Biphenyl)-1h-Benzoimidazole -5-Carboxamidine	-6.12
N,7-diphenyl-2,4,8-triaza-5-azoniabicyclo [3.3.0]octa-3,7,9-triene-2,3-diamine	-6.1
1-Methyl-5-(2-phenoxymethyl-pyrrolidine -1-sulfonyl)-1h-indole-2,3-dione	-6.1
Morphine	-6.04
Bucladesine	-6.03
Clocapramine	-6.02
N-Hydroxy-2-[4-(4-Phenoxy- Benzenesulfonyl)-Tetrahydro-Pyran-4-Yl] -Acetamide	-6.02
Ouabain	-6.02

between ligand and protein (Shityakov and Forster, 2014). In our study, a total of 22 compounds derived from the CSR1 isolate qualified as per the threshold for binding affinity effectively in neutralizing the TMV CP (Table 1). The binding affinities of these compounds from CSR1 ranged from -6.02 kcal/mol, as observed for Ouabain to -7.30 kcal/mol, exemplified by Penitrem A. Notably, two specific compounds, Penitrem A with a binding affinity of -7.30 kcal/mol and Milbemycin A4 with -7.22 kcal/mol, demonstrated the highest binding affinities among the compounds tested.

Visualization of TMV CP and CSR1 Metabolites Interaction

The 3D and 2D visualization of top three phytochemicals alongside the positive control ningnanmycin based on their binding affinity with TMV CP are represented in Fig. 3 and Fig.4, respectively. The 3D visualization represents the exact location or the binding pocket of the target protein, whereas the 2D structure visualization represents the different bonds formed between the amino acid residues of the viral target protein and the ligand. These visualizations collectively provide a comprehensive perspective on the molecular interactions that underlie the potential antiviral activity of the identified metabolites. The major contributor in the dock score is the hydrogen bonds formation between the ligand structures and viral receptors which is responsible for inhibiting the target protein and it reflects the firmness of bonding between the protein and ligand (Chen *et al*., 2016). Ligand

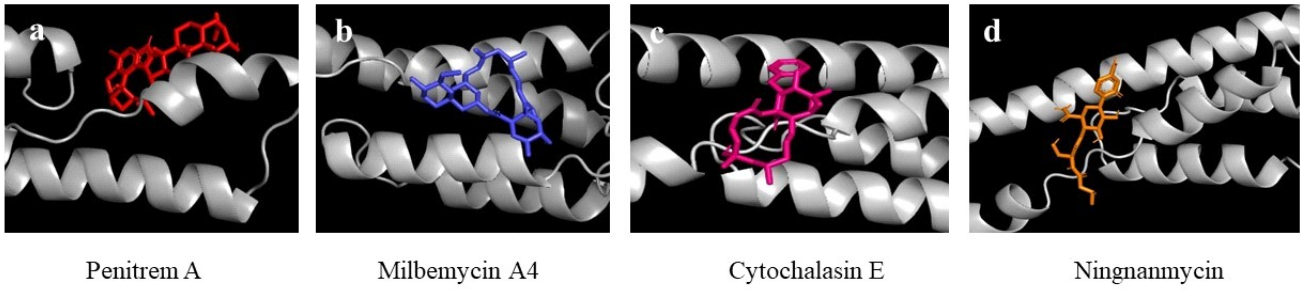


Fig. 3 : The 3D visualization of interaction between TMV CP (Tobacco mosaic virus coat protein) with ningnanmycin (positive control) and representative metabolites from CSR1. The ligand-binding pose showing highest binding affinity with least root mean square deviation (RMSD) was selected. The protein–ligand interaction in 3D structures were visualized in PyMOL

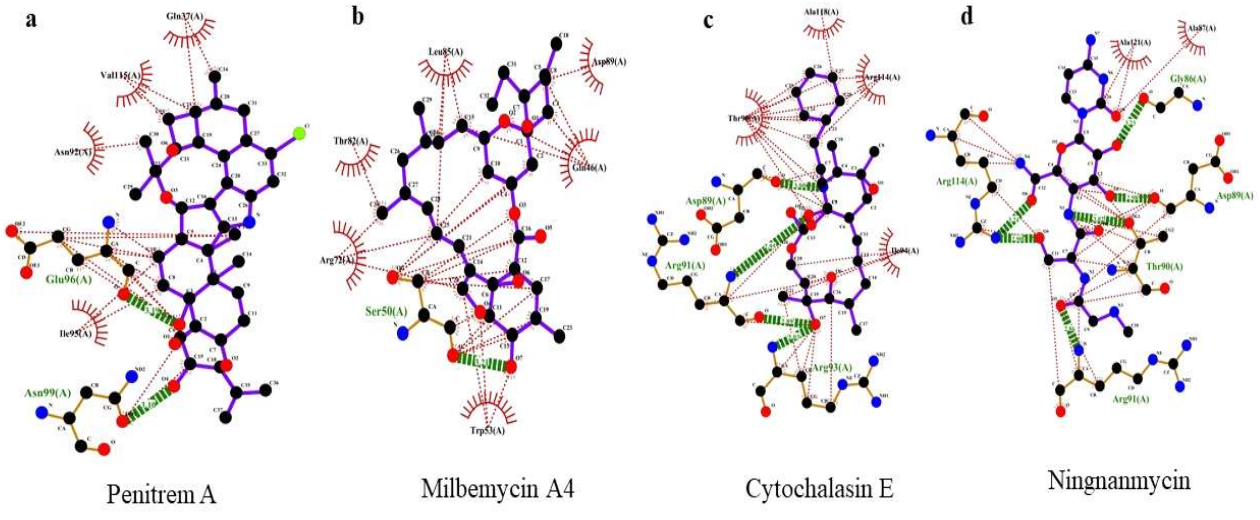
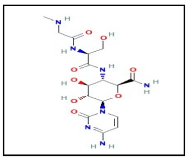
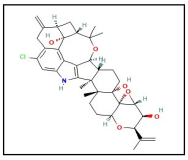
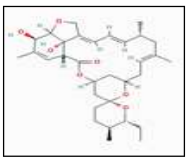
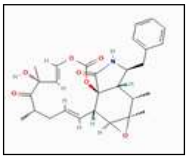
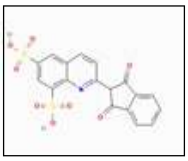
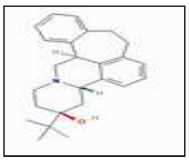


Fig. 4 : 2D visualization of interaction between TMV CP (Tobacco mosaic virus coat protein) with ningnanmycin (positive control) and representative metabolites from endophyte CSR 1

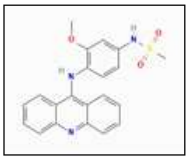
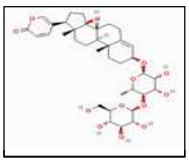
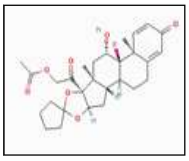
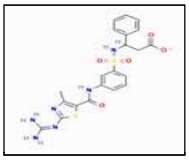
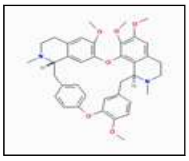
TABLE 2

Number of hydrogen bonds formed during the interaction between CSR1 endophyte metabolites and ningnanmycin (positive control) with TMV CP

Compounds and PubChem ID	Structural and chemical formula	Number of hydrogen bonds formed during interaction	Amino acid residues of viral receptor involved in hydrogen bonding with ligand
Ningnanmycin (Positive Control) 44588235	 $C_{16}H_{25}N_7O_8$	6	GLY86, ASP89, THR90
Penitrem A 6610243	 $C_{37}H_{44}ClNO_6$	2	GLU96, ASN99
Milbemycin A4 9959038	 $C_{32}H_{46}O_7$	1	SER50
Cytochalasin E 5458385	 $C_{28}H_{33}NO_7$	4	ASP89, ARG91, ARG93
2- (1,3-Dioxo-2,3-dihydro-1 h-inden-2-yl) quinoline-6, 8-disulfonic acid 24672	 $C_{18}H_{11}NO_8S_2$	2	ASP116, GLN37
(+) - Butaclamol 37459	 $C_{25}H_{31}NO$	1	GLY86

Continued....

TABLE 2 Continued....

Compounds and PubChem ID	Structural and chemical formula	Number of hydrogen bonds formed during interaction	Amino acid residues of viral receptor involved in hydrogen bonding with ligand
Amsacrine 2179	 $C_{21}H_{19}N_30_3S$	1	ASP89
Scillaren A 441870	 $C_{36}H_{52}O_{13}$	7	ASP110, ASP117, ARG91, ARG114, ASN92, GLU107, SER124
Amcinonide 443958	 $C_{28}H_{35}FO_7$	2	ASN99, GLN39
3-[[3-[[2-(diaminomethylideneamino) -4-methyl-1, 3-thiazole -5-carbonyl] amino]phenyl] sulfonylamino] -3-phenylpropanoate 18322725	 $C_{21}H_{21}N_6O_5S_2$-	2	ASP116, ASN92
Tetrandrine 73078	 $C_{38}H_{42}N_2O_6$	1	THR38

structures and necessary hydrogen bond formation between CSR1 metabolites with TMV CP has been illustrated in Table 2. This data offers critical insights into the molecular interactions that underlie the potential inhibition of the target protein, shedding light on the mechanisms through which these compounds exert their antiviral effects.

Thus, the *in-silico* analysis of the TMV CP and the metabolites derived from the CSR1 endophyte has revealed strong binding affinities, indicating the potential of these endophyte metabolites as candidates for the development of anti-TMV drugs. However, it's essential to emphasize that *in-silico* predictions serve as the initial step in the drug development

process. To validate these findings and progress toward effective anti-TMV drug development, *in-vivo* evaluations are necessary. These studies will provide critical insights into the practicality and safety of utilizing these endophyte metabolites as therapeutic agents against TMV, contributing to advancements in the field of plant virology and antiviral drug discovery.

References

Biovia, D. S., 2020, Discovery studio visualizer, Release 2020, San Diego: Dassault Systemes.

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M. and Savidge, T. C., 2016, Regulation of protein-ligand binding affinity by hydrogen bond pairing. *Sci. Adv*., **2** (3) : 1501240.

Cournia, Z., Allen, B. and Sherman, W., 2017, Relative binding free energy calculations in drug discovery: recent advances and practical considerations. *J. Chem. Inf. Model*, **57** (12) : 2911 - 2937.

Degtyarenko, K., De Matos, P., Ennis, M., Hastings, J., Zbinden, M., Mcnaught, A., Alcantara, R., Darsow, M., Guedj, M. and Ashburner, M., 2007, ChEBI:

A database and ontology for chemical entities of biological interest. *Nucleic Acids Res*, **36** : 344 - 350.

Delano, W. L., 2009, The PyMOL Molecular Graphics System *http://* www.pymol. *org*.

Gunatilaka, A. A., 2006, Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity and implications of their occurrence. *J. Nat. Prod*., **69** (3) : 509 - 526.

Hanwell, M. D, Curtis, D. E, Lonie, D. C., Vandermeersch, T., Zurek, E. and Hutchison, G. R., 2012, Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J. Chem. Inf. Mode.,* **4** (1) : 1- 17.

Hiremath, S. S., Vinaykumar, H. D., Koti, P. S., Nandan, M., Shankarappa, K. and Reddy, C. N. L., 2021, In-silico investigation of cucumber *Mosaic virus* (CMV) resistance genes and transcription factors involved in CMV hosts generated from Chilli-CMV transcriptome data. *Mysore J. Agric. Sci*., **55** (4) : 355 - 363.

Ibrahim, A., Odon, V. and Kormelink, R., 2019, Plant viruses in plant molecular pharming: toward the use of enveloped viruses. *Front. Plant Sci.*, **10** : 803.

Jones, R. A., 2021, Global plant virus disease pandemics and epidemics. *Plants*, **10** (2) : 233.

Kanehisa, M. and Goto, S., 2000, KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.,* **28** : 27 - 30.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A. and Wang, J., 2016, PubChem substance and compound databases. *Nucleic Acids Res.,* **44** : 202 - 213.

Laskowski, R. A. and Swindells, M. B., 2011, LigPlot: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model*., **51** (10) : 2778 - 2786.

Laskowski, R. A., Macarthur, M. W., Moss, D. S. and Thornton, J. M., 1993, PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr*., **26** (2) : 283 - 291.

Li, Z., Wen, W., Qin, M., He, Y., Xu, D. and Li, L., 2022, Biosynthetic mechanisms of secondary metabolites promoted by the interaction between endophytes and plant hosts. *Front. Microbiol*., **13** : 1 - 15.

Likhith, R. and Peter, A., 2023, Comparative in silico Analysis of Coat Protein (CP) of Tomato Leaf Curl Virus (ToLCV) and Tomato Yellow Leaf Curl Virus (TYLCV) and their molecular docking with GroEL protein of *Hamiltonella* an Endosymbiont of their vector bemisia tabaci. *Mysore J. Agric. Sci.*, **57** (2) : 195 - 204.

Linnakoski, R., Reshamwala, D., Veteli, P., Cortina-escribano, M., Vanhanen, H. and Marjomaki, V., 2018, Antiviral agents from fungi: Diversity, mechanisms and potential applications. *Front. Microbiol.,* **9** : 1 - 18. https://doi.org/10.3389/fmicb.2018.02325.

Liigand, P., Kaupmees, K., Haav, K., Liigand, J., Leito, I., Girod, M., Antoine, R. and Kruve, A., 2017,

Think negative: finding the best electrospray ionization/MS mode for your analyte. *Anal. Chem*., **89** (11) : 5665 - 5668.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J., 2009, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput Chem.,* **30** (16) : 2785 - 2791.

Nandan, M., 2021, Studies on fungal endophytes against soil-borne fungal pathogens of tomato *Ph. D. Thesis* (Unpub.), *Univ. Agric. Sci*., Bangalore., 1 - 18.

Natesh, J., Mondal, P., Kaur, B., Salam, A.A.A., Kasilingam, S. and Meeran, S. M., 2021, Promising phytochemicals of traditional Himalayan medicinal plants against putative replication and transmission targets of SARS-CoV-2 by computational investigation. *Comput. Biol. Med*., **133** : 104383.

Pappas, N., Roux, S., Holzer, M., Lamkiewicz, K., Mock, F., Marz, M. and Dutilh, B. E., 2021, Virus bioinformatics. Encyclopedia *Virol*., **51** : 124 - 135.

Pansanit, P. and Pripdeevech, P., 2018, Antibacterial secondary metabolites from an endophytic fungus, *Arthrinium* sp. MFLUCC16-1053 isolated from *Zingiber cassumunar*. *Mycol.*, **9** (4) : 264 - 272.

Peters, L. P., Prado, L. S., Silva, F. I. N., Souza, F. S. C. and Carvalho, C. M., 2020, Selection of endophytes as antagonists of *Colletotrichum gloeosporioides* in acai palm. *Biol. Control*, **150** : 104350.

Pitt, J. J., 2009, Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clin. Biochem. Rev.*, **30** (1) : 19 - 25. Pluskal, T., Castillo, S., Villar-briones, A. and Oresic, M., 2010, MZmine 3 : modular framework for processing, visualizing and analyzing mass spectrometry-based molecular profile data. BMC bioinform., **11** (1) : 1 - 11.

Ravikumar, A., Ramakrishnan, C. and Srinivasan, N., 2019, Stereochemical assessment of (ϕ, ψ) outliers in protein structures using bond geometry-specific Ramachandran steric-maps. Structure, **27** (12) : 1875 - 1884.

Shityakov, S. and Forster, C., 2014, In silico predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. *Adv. Appl. Bioinform. Chem. AABC.*, **7** : 23.

Shode, F. O., Idowu, A. S. K., Uhomoibhi, O. J. and Sabiu, S., 2021, Repurposing drugs and identification of inhibitors of integral proteins (Spike protein and Main protease) of SARS-CoV-2. *J. Biomol. Struct. Dyn.*, **40** : 6587 - 6602. doi:10.1080/07391102.2021.1886993.

Tang, L., Shang, J., Song, C., Yang, R., Shang, X., Mao, W., Bao, D. and Tan, Q., 2020, Untargeted metabolite profiling of antimicrobial compounds in the brown film of Lentinula edodes mycelium *via* LC-MS/MS analysis. *ACS omega*, **5** (13) : 7567 - 7575.

Trott, O. and Olson, A. J., 2010, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.* **31** (2) : 455 - 461.

Wang, Y., He, F., Wu, S., Luo, Y., Wu, R., Hu, D. and Song, B., 2020, Design, synthesis, anti-TMV activity, and preliminary mechanism of cinnamic acid derivatives containing dithioacetal moiety. *Pestic. Biochem. Physiol.*, **164** : 115 - 121.

Xia, R., Guo, T., Chen, M., Su, S., He, J., Tang, X. and Xue, W., 2019, Synthesis, antiviral and antibacterial activities and action mechanism of penta-1, 4-dien-3-one oxime ether derivatives containing a quinoxa line moiety. *New Journal of Chemistry*, **43** (42) : 16461 - 16467.

Zhang, Y. and Skolnick, J., 2005, TM-align: a protein structure alignment algorithm based on the TM-score. *Nucleic Acids Res*., **33** (7) : 2302 - 2309.

Zhao, L., Feng, C. H., Hou, C. T., Hu, L. Y., Wang, Q. C. and Wu, Y. F., 2015, First discovery of acetone extract from cottonseed oil sludge as a novel antiviral agent against plant viruses. *PLoS ONE*, **10** : e0117496.