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In silico screening identifies Daphnodorin-C as a potential inhibitor of the PMK1 pathway in the management of rice blast disease

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Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating threats to global food security, especially in nations where rice is staple food. Despite the availability of chemical fungicides, the emergence of resistant fungal strains necessitate the search for alternative, natural and environment friendly solutions. In this study, we utilized *in silico* screening approaches to identify potential inhibitors targeting the PMK1 pathway, a critical regulator in the appressorium formation and fungal infection. Natural compounds from economically important plants of south Asia available in NPASS database were screened against the target PMK1 protein. In the screening Daphnodorin-C from *Daphne odora* was identified as a promising candidate, with a docking score of -10.9 kcal/mol. Molecular dynamics simulations (MDS) confirmed the stability of the Daphnodorin-C–PMK1 complex over 100 ns simulation period, with root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) values within permissible limits. Daphnodorin-C exhibited moderate toxicity against *T. pyriformis* and minnows, suggesting moderate effect on aquatic life if contaminated. It's *in silico* safety profile shows no hepatotoxicity or skin sensitization, limited skin permeability, though with mild immune and respiratory effects. These findings suggest that Daphnodorin-C could be a promising lead compound for rice blast disease management.

Keywords: *Daphne odora*, *Magnaporthe oryzae*, Mitogen activated protein kinase, Molecular docking, Molecular dynamics simulation, Natural compounds, NPASS database, Rice blast fungus

Magnaporthe oryzae, a fungus that causes rice blast disease, is one of the most destructive pathogens affecting rice (*Oryza sativa*), a staple food for over half the world's population[1,2]. Under favorable conditions, this disease can lead to significant damage, resulting in 10-30% of annual rice yield loss[3,4]. The fungus infects rice plants at all developmental stages and attacks various parts, including leaves, stems, nodes, and panicles[5].

It produces special structures called conidia, which are three-celled, teardrop-shaped spores formed on aerial stalks in a specific pattern. These conidia stick to the surface of the leaves using a glue-like substance which results in the initiation of the infection cycle[6]. After attaching to the surface of the leaf the conidia germinate, producing germ tubes that eventually form a specialized structures called appressoria[5]. Due to melanization and accumulation of glycerol these appressoria become rigid and strong and develop a thick layer. This process generates high pressure, which allows the fungus to break through the plant's outer cell wall initiating the infection process[7].

Once penetrated, the fungus spreads using hyphae which are invasive fungal extensions that grow in the intracellular spaces. The movement of the hyphae between the cells is facilitated by plant cells intracellular connections called plasmodesmata[8]. These interactions between the fungal hyphae and the plant's cell membranes are crucial for the infection spread[9]. The built up of pressure in the appressorium is necessary for the fungus to break through the tough cell walls. A key regulator of this process is the protein PMK1, in Mitogen-activated protein kinase (MAPK) pathway**,** which controls the expression of genes needed for appressorium formation[10]. Strategies can be developed to disrupt this pathway which prevents the appressorium from penetrating the cell wall thereby preventing the spread of the infection within the plant. Therefore, PMK1 can be a potential target for designing new inhibitor molecules for controlling rice blast disease. Blocking PMK1 could limit the damage caused by this destructive pathogen, which threatens global rice production and food security.

Previous extensive studies have highlighted the role of PMK1 (MAPK) in the formation of appressorium which initiates the spread of the fungus. A functional PMK1 is essential for the pathogenicity

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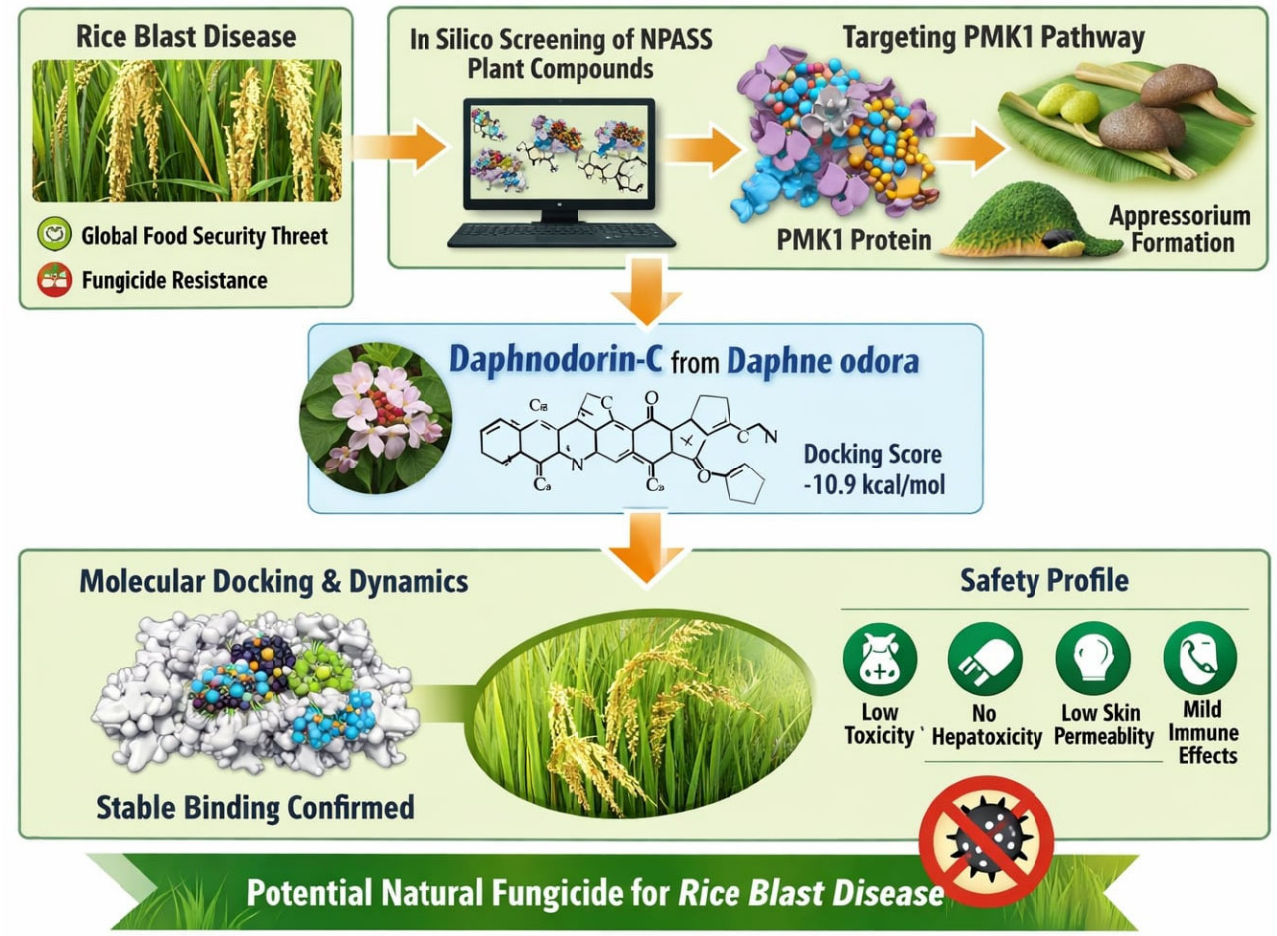

Graphical abstract

and virulence of rice blast fungus, making it one of the potential targets for developing antifungal compounds. Understanding the molecular mechanisms underlying PMK1's function in the MAPK pathway can lead to innovative approaches for fungal management in crops[11]. The rich diversity of plant derived bioactive compounds deposited in databases form a reservoir for screening for potential molecules which can be used as effective antifungal agents. The availability of natural compound databases enables researchers in harnessing natural resources for agricultural and medicinal applications.

The exploration of various plant based natural bioactive compounds has revealed a many effective antimicrobial agents, particularly against fungal infections. These compounds are often extracted using different solvents such as ethanol, methanol, and dichloromethane, from various plant tissues like leaves, roots, bark etc. Notably, many plant extracts have demonstrated antifungal activities, providing promising alternatives to synthetic molecules and contributing to the development of sustainable natural antifungal stratagies[12]. Many online repositories were created to store organic molecules structures. One among is the Natural Product Activity and Species Source (NPASS) database which has nearly 94000 natural compounds predominantly from economically important plants of Asia. This database was used in this study to screen bioactive compounds from plant sources which have the potential in combating resistant *Magnaporthe* strains[13].

Materials and Methods

Protein preparation

The three-dimensional (3D) structure of Mitogen-activated protein kinase PMK1, UniProt ID G4N0Z0 (PMK1_PYRO7), was retrieved from the UniProt database (https://www.uniprot.org/). To ensure the structural integrity and quality of the model, structural evaluation was done using computational tool

PROCHECK to assess the stereo chemical quality of the protein structure by analyzing the Ramachandran plot, which highlights the dihedral angles of amino acid residues and their compatibility with ideal values[14]. Additionally, the ERRAT analysis was used to evaluate the overall quality factor of the structure, identifying regions of potential error based on statistical comparisons with available confirmed high-resolution structures[15]. Together, these analyses provided a comprehensive assessment of the protein model's reliability and accuracy, ensuring its suitability for further functional and computational studies (https://saves.mbi.ucla.edu/).

Ligand preparation

Ligands for this study were sourced from the NPASS database which is a comprehensive repository of natural products and their bioactivities[16]. 4500 compounds from known economically important plants of Indian sub-continent available in the NPASS database were downloaded for screening against the target protein, Mitogen-activated protein kinase PMK1. To facilitate molecular docking and further computational analyses, the ligands were initially obtained in Structure Data File (SDF) format from NPASS database, a standard format for representing chemical structures. Open Bable software was used to convert the SDF files into the Protein Data Bank (PDB) format, which is compatible with most molecular docking software[17].

Identification of active site

The docking grid was meticulously designed to ensure accurate simulation of the ligand-binding process with Mitogen-activated protein kinase, PMK1. CASTp3.0 server was used to identify the boundaries and critical residues of PMK1 active site[18]. CASTp3.0 analyzes protein structures to detect and measure pockets and cavities that are likely to serve as functional sites of a protein. This tool provided a detailed insight into the geometry and dimensions of the docking grid which can be effectively accessed by the ligand improving the accuracy and reliability of the docking simulations (https://cfold.bme.uic.edu/castp-fold/).

Molecular docking

Molecular docking studies were performed using PyRx, a virtual screening tool that uses a powerful computational algorithm. PyRx leverages the robust and widely used AutoDock algorithm to predict the binding affinities of ligands within the active site of the protein[19]. The AutoDock algorithm works on a grid-based energy evaluation method, which calculates the binding free energy of ligands at various positions and conformations within the docking grid of the protein. This approach gives both precision and reliability in identifying the most favorable ligand-protein interactions.

In the process of docking, the software, PyRx was used which optimized the protein and ligands structures by ensuring the correct format and charge orientation. The grid in which the process of binding was facilitated had been set to the coordinates and dimensions, appropriate to the active site of Mitogen-activated protein kinase PMK1. The algorithm assessed the poses of binding on the basis of the binding energy scores.

The PyRx program, together with the AutoDock algorithm, remarkably allowed for high-throughput docking that was accurate and helped to identify potential ligands by ranking them based on highly favorable binding affinities and specificity.

Molecular dynamics simulations

Desmond (Schrodinger) package was used to perform Molecular Dynamic Simulation for duration of 100 nano seconds. Protein ligand complex was retrieved from docking studies to perform Molecular Dynamic simulation. As docking studies provides only a static view of ligand binding in active site, Molecular dynamic simulation studies are required to understand the stability of docking under various physiological conditions. MDS tend to compute the atoms movement with time by integrating Newton's classical equation of motion. Protein preparation wizard was used to pre process the protein ligand complex, which also included optimization and minimization of the complex. All systems were prepared by the System Builder tool. Solvent Model with an orthorhombic box was selected as TIP3P (Transferable Intermolecular Interaction Potential 3 Points). The OPLS_2005 force field was used in the simulation[20]. The models were made neutral by adding counter ions where needed. To mimic the physiological conditions, 0.15 M salt (NaCl) was added. The NPT ensemble with 300 K temperature and 1 atmospheric pressure was selected for complete simulation. The trajectories were saved after every 10 ps for analysis, and the stability of simulations was evaluated by calculating the root mean square deviation (RMSD) of the protein and ligand over time.

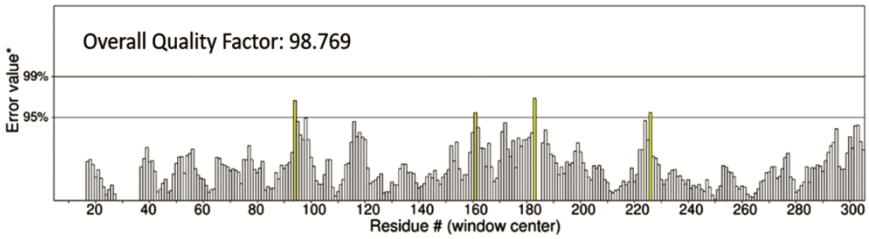

Fig. 1 — ERRAT analysis of the PMK1 protein structure showing an overall quality factor of 98.769, indicating high model reliability

Analysis of fungicide-like properties

To ensure a potential compound possesses, the biochemical characteristics of an effective fungicide, early evaluation during fungicide discovery is crucial in minimizing the risk of quick development of resistance in fungi. The physicochemical properties of candidate compounds were analyzed to determine their fungicide-like nature. Since no specific criteria exist for fungicides, we evaluated the natural compounds' fungicide-likeness using the well-established drug-likeness principle, Lipinski's Rule of Five. The ADMETlab2.0 server (https://admetlab3.scbdd.com) was employed to assess the fungicidal properties of the top metabolites, providing predictions based on their characteristics in relation to Lipinski's Rule[21].

Toxicity analysis

The pkCSM web-based server (https://biosig.lab.uq.edu.au/pkcsm/) was used to forecast the General toxicity of the identified compounds[22]. This tool relies on graph-based signatures to analyze molecular distance patterns, making it useful for predicting pharmacokinetic features.

Results

PMK1 Structure analysis

The structural evaluation of the Mitogen-activated protein kinase PMK1 model was conducted using rigorous validation tools to ensure its suitability for computational studies. The quality factor, as determined by ERRAT analysis, was 98.76% indicating high level of structural conformity and stability. ERRAT evaluates the quality of the protein structure by analyzing non-bonded atomic interactions and comparing them to established high-resolution protein structures. A quality factor of 100% of the modelled protein indicates a highly reliable model with minimal errors, showcasing the structural integrity of PMK1 model (Fig. 1).

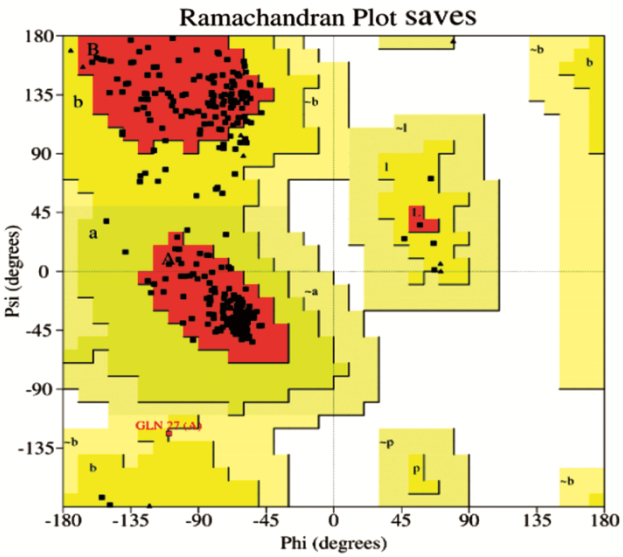

Fig. 2 — Ramachandran plot of Mitogen-Activated Protein Kinase (PMK1) illustrating residues in the most favored regions (A, B, L) and additionally allowed regions (a, b, l, p), indicating good stereochemical quality of the protein model

When phi (ϕ) and psi (ψ) dihedral angles of the amino acid residues were analyzed using PROCHECK based on the Ramachandran plot, it revealed that 86.6% of the amino acid residues fell in the core favorable regions indicating a well-constructed backbone conformation, typical of high-quality protein structure. An additional 13.1% of residues were found in the allowed region of the Ramachandran plot which are acceptable but less frequent in ideal protein geometries. Only 0.3% of residues were in the generously allowed regions, which are considered marginal but still permissible. Notably, there were no residues (0.0%) in the disallowed region, implying the absence of any significant structural anomalies or unfavorable stereochemistry in the predicted protein structure (Fig. 2 & Table 1).

Active site determination for PMK1

CASTp3.0 generated a grid by creating a docking box centered at specific coordinates (1.3335, 1.0821,

Table 1 — Distribution of amino acids within the Ramachandran plot

Ramachandran plot statistics		
Residues in most favoured regions [A, B, L]	278	86.6%
Residues in additional allowed regions [a, b, l, p]	42	13.1%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	1	0.03%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	321	100%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	12	
Number of proline residues	21	
Total number of residues	356	

The table shows the percentage of residues in the most favored region (A, B, L) and additionally allowed regions (a, b, l and p) and location of glycine, Proline residues

Table: 2 — Top 5 Scored compounds by Docking based screening from the NPASS Database and their Source organisms.

S. No	Compound Name	Binding Affinity Kcal/mol	Source Organism
01	Daphnodorin- C	-10.9	*Daphne odora*
02	Picrasidine	-10.0	*Picerasma quassiodes*
03	Mesylate	-9.8	*Cannabis sativa*
04	Cynadine 3-5 glucoside	-9.5	*Punica grantum*
05	Sesamin	-9.24	Fagara plant

and 3.7045) which precisely align with the PMK1's active site. This spatial orientation was necessitated to be determined based on structural data to target the key functional region of the protein effectively in order to deactivate the protein. Based on the result, the dimensions of the docking box were set to 25 Å × 25 Å × 25 Å along the X, Y, and Z axes, providing a sufficiently large volume to accommodate the entire active site and potential ligand-binding regions. This size ensured that the docking simulation accounted for the flexibility in ligand orientation and positioning, which enabled thorough screening of binding interactions (Fig. 3).

Molecular docking

Molecular docking studies were conducted to identify potential ligands with strong binding affinities for PMK1 from among the NPASS database. Among the screened compounds of economically important plants found in South Asia, Daphnodorin-C, a spiro-biflavanoid from *Daphne odora*, emerged as a highly promising candidate with a docking score of -10.9 Kcal/Mol (Table 2). Top five ligands with highest docking scores were given in the (Table 2). The docking simulation yielded an impressive docking score of -10.9, for Daphnodorin-C indicating a strong binding interaction and high thermodynamic favorability. Daphnodorin-C was found to form stable interactions with several key amino acid residues in the predicted PMK1active site.

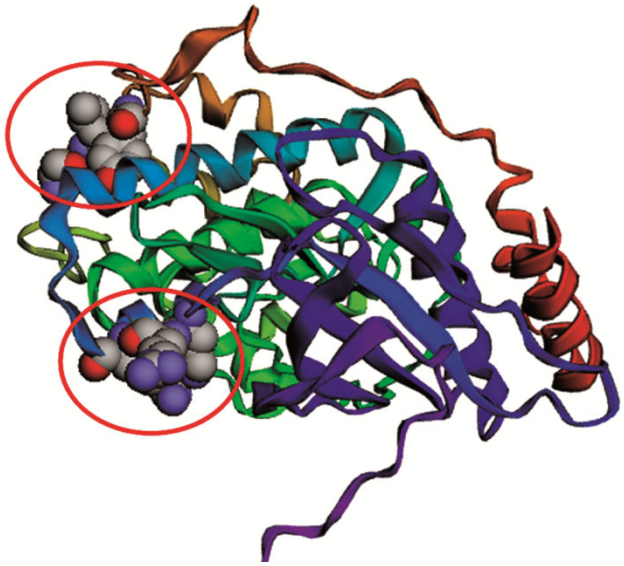

Fig. 3 — Predicted active site and binding pockets of PMK1 protein highlighted in red circles, as identified using the CASTp 3.0 online tool

Precisely, the compound interacted with VAL 30, ALA 34, GLU 32, VAL 38, LYS 53, TYR 35, ASP 166, CYS 165, SER 152, LEU 155, ASP 110, and ALA 51 residues of PMK1 protein (Fig. 4). These interactions included a combination of hydrogen bonds, Vander Waals forces, and hydrophobic interactions, which collectively contributed to the ligand's stable binding within the binding pocket of the protein.

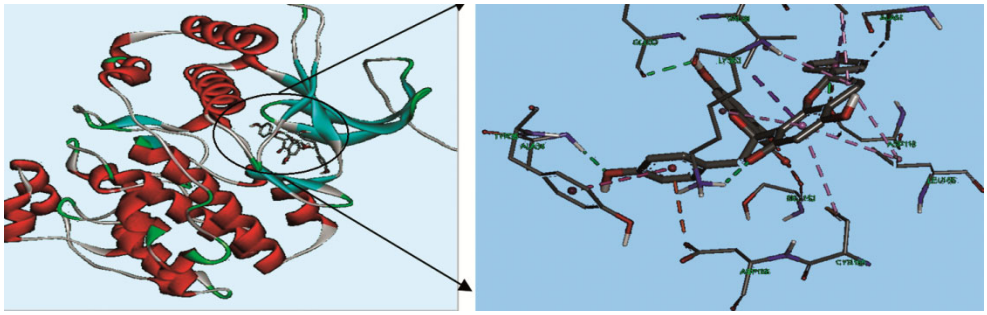

Fig. 4 — Molecular docking interaction of Daphnodorin-C with PMK1, showing the binding pattern and key interacting amino acid residuesVAL 30, VAL 38, GLU 32, LYS 53, ALA 34, TYR 35, ASP 166, CYC 165, SER 152, LEU 155, ASP 110, ALA 51

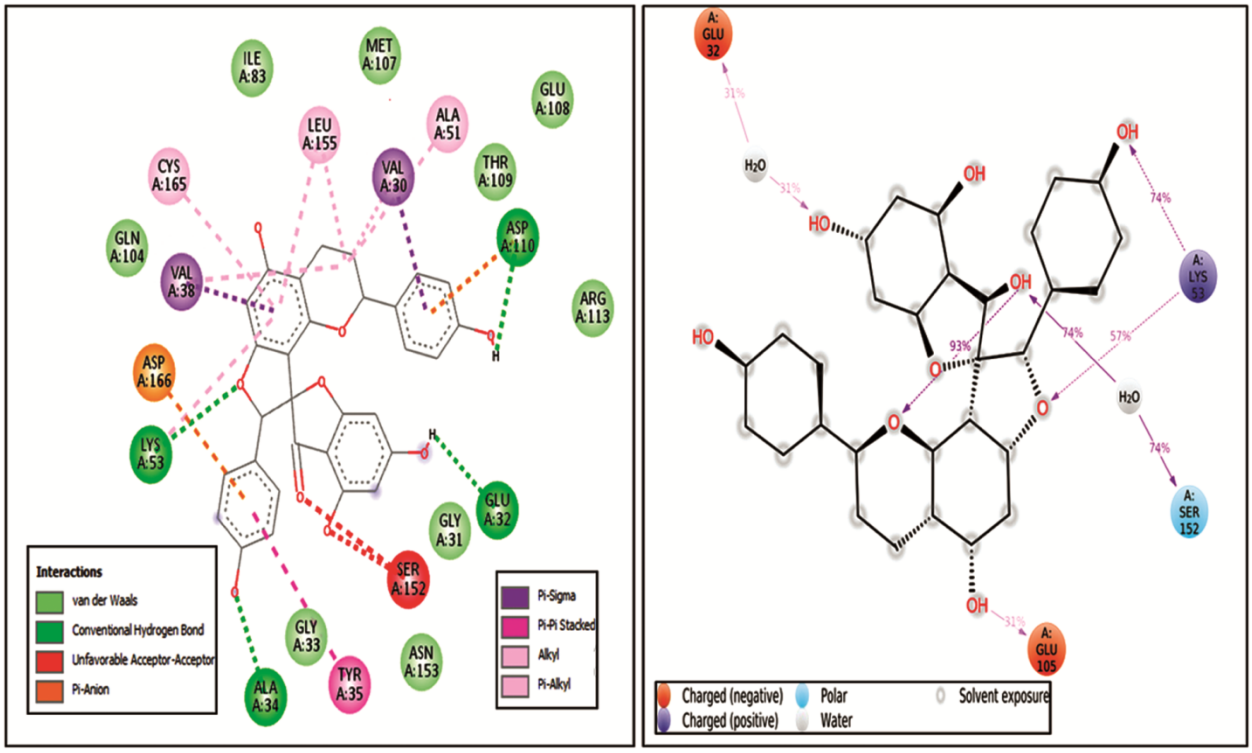

Fig. 5 — Two-dimensional interaction diagram of Daphnodorin-C with PMK1 at 0 ns (left) and 100 ns (right), showing molecular interactions such as hydrogen bonds, van der Waals forces, π-π stacking, and electrostatic interactions. Main residues involved in ligand stabilization within the binding site are showed

From all the ligand interacting amino acids in PMK1, three residues VAL 30, VAL 38, and LYS 53 are active site amino acids crucial for the binding of Daphnodorin-C. The interaction of Daphnodorin-C with these amino acids can potentially results in inhibition of PMK1 activity. The specific attachment of these residues suggests that Daphnodorin-C has a strong affinity towards the PMK1 active site, making it a strong candidate for further biochemical and anti-fungal experimental validations. The ability of Daphnodorin-C to engage multiple residues within the binding pocket suggests its potential as a lead molecule for deriving fungicide targeting PMK1. These findings provide a strong base for further experimental validation and structural optimization to enhance its fungicidal potential (Fig. 5).

Molecular dynamics simulation:

The molecular dynamic simulations at 100 ns shows the stability and binding efficacy of the Daphnodorin-C and PMK1 individually and in complex, as shown by RMSD and protein-ligand interaction analyses. The RMSD analysis denotes structural stability of the protein, with fluctuations

within the acceptable range (1–3 Å) during the 100 ns simulation, suggesting equilibration (Fig. 6). RMSF indicates flexible regions in loops and rigid areas in helices and strands, confirms integrity. Secondary structure analysis confirms 28.2% helices and 12.68% strands, leads to the overall integrity and stability of the protein. Ligand RMSD showed stable binding within the pocket during the simulation time, while RMSF identified minimal fluctuations in the key regions,

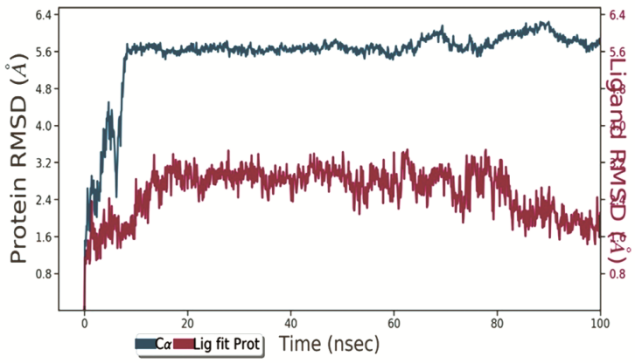

Fig. 6 — Root Mean Square Deviation (RMSD) of the protein-ligand complex over 100 ns, indicating the structural stability of the PMK1–Daphnodorin-C complex throughout the MD Simulation

indicating stable conformations. The torsional profile reflects Daphnodorin-C's adaptability to the binding environment with manageable conformational strain. Stable interaction was observed to be the result of hydrogen bonds, hydrophobic forces, and water bridges between protein and ligand (Fig. 7). High-frequency interactions (>30% simulation time) underline key residues in ligand binding and a docking score of -10.9 confirms strong binding affinity, further validating the MDS results. The MDS results confirm the stability of the PMK1-Daphnodorin-C complex. Minute protein RMSD, consistent ligand RMSD, and favorable interaction patterns spotlight the compatibility of Daphnodorin-C as a potential inhibitor. The observed binding and structural dynamics make Daphnodorin-C a promising candidate against *Magnaporthe* targeting PMK1.

Analysis of fungicide-like properties

The fungicidal properties of a compound against a plant pathogen can be assessed through its toxicity parameters. When Daphnodorin-C was analyzed using ADMET, the compound exhibited moderate toxicity towards *T. pyriformis* (log µg/L = 0.285) and potential environmental toxicity (Minnow toxicity log

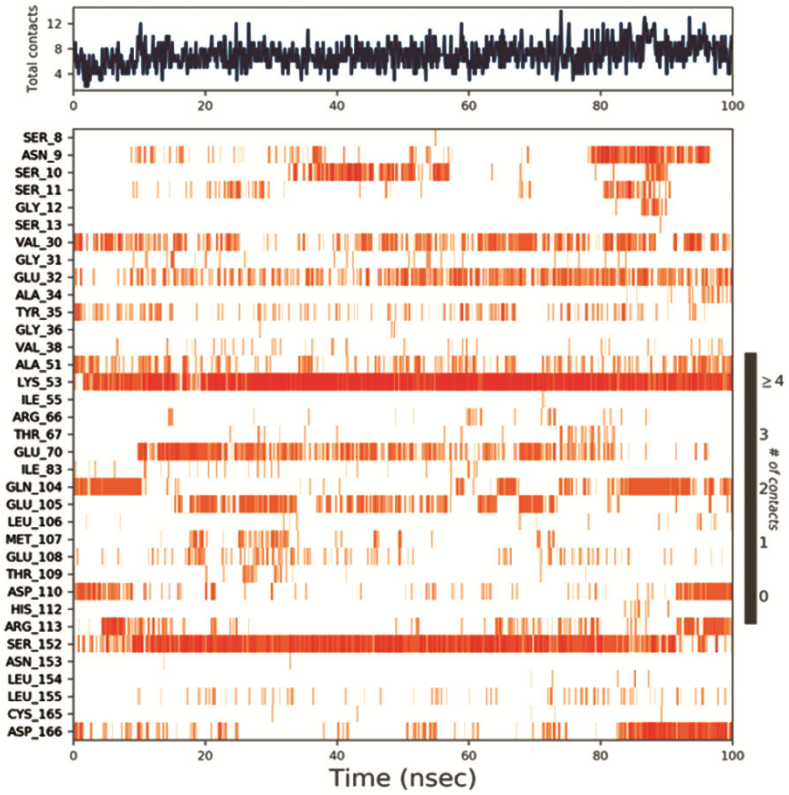

Fig. 7 — Timeline representation of protein-ligand interactions over the 100 ns simulation. The above blue shows the total number of specific contacts formed with the ligand, while the below panel represents the persistence of interactions for individual residues of trajectory frames. Darker orange indicates stronger or multiple interactions based on the contact frequency

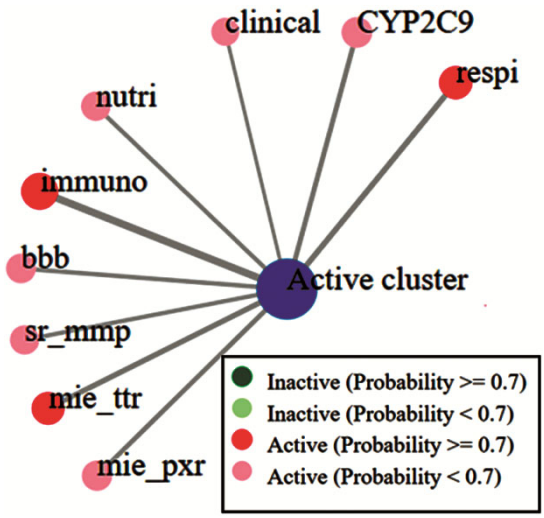

Fig. 8 — *In silico* toxicity prediction of Daphnodorin-C using ADMETlab 3.0. The molecule exhibited mild respiratory and immunotoxic effects

mM = 1.755). Though the toxicity is moderate, a dosage study to minimize the environmental damage and at the same time effective against the fungus need to be done to fix the concentration of spray. If targeting plant pathogens, its efficacy should be evaluated through antifungal bioassays to confirm its inhibitory effects on fungal growth

Evaluation of compound toxicity

The compound showed moderate toxicity to *Tetrahymena pyriformis* (0.285 µg/L) and minnows (1.755 mM), suggesting possible environmental effects at higher doses. Oral Toxicity in Rats exhibited moderate toxicity, with an acute LD50 of 2.382 mol/kg and a chronic LOAEL of 1.092 mg/kg/day. A low max tolerated dose (0.286 mg/kg/day) means it may work effectively at smaller doses. Safety Profile of Daphnodorin-C exhibited no hepatotoxicity or skin sensitization, making it potentially safe for mammals and plants. It's Absorption (ADME) is 100%, absorbed in the intestines but through the skin. It stays mostly in the bloodstream, not reaching the brain. Not broken down by key liver enzymes but may interact with some drugs. Excretion clears out at a moderate rate. The compound showed **a** balanced safety and toxicity profile. However, experimental testing is needed to confirm its real-world effectiveness and safety (Fig. 8).

Discussion

Flavons are phenolic structures containing one carbonyl group, and have been found to have anti-microbial activity against a wide range of fungi and bacteria. Their ability to complex with extra cellular and soluble proteins and their lipophilic nature makes them effective anti-fungal candidates[22]. Flavonoids isolated from bark of *Erythrina burtii,* and rhizome of *Alpine officinarum* have shown anti-fungal activity against a variety of fungal pathogens[23,24].

New anti-microbial agents with improved bioavailability and anti-fungal & anti-bacterial properties were developed using derivatives from natural Flavones. A flavanol analogue replacing phenyl ring with hetero aromatic system and various substituents on chromone system is synthesized by Ashok D *et. al*, and tested against several fungal strains[25]. These synthesized flavanol derivatives exhibited good anti-fungal activity at 50 µg/ml concentration against several fungal strains (*Asperigillus niger, Pencellium italicum, Fusarium oxysporum*)[26]. In a study, Lv X. H. *et. al* synthesized derivatives from natural flavones such as baicalein, lutolin, qurcetol, apigenin and kaempferol as precursors by applying mannich reaction at position 8 of the chromone moiety. These derivatives have shown substantive inhibition against bacterial topoisomerase II & topoisomerase IV[27].

The genus *Daphne Linn* is the most diverse genus in the Thymelaeaceae family. Many species from this genus are source of Spiro Biflavanoids and other phyto compounds with bioactive properties. These phytochemicals have been demonstrated to possess a variety of biological activities including anti-cancer, anti-HIV and anti-inflammatory effects[28]. Several *Daphne* plants have been used in many Asian countries in traditional medicine for treatment against inflammation and Rheumatism. Diterpenoids from the flower buds of *D.genkwa* has been shown to have pesticidal properties and used as pesticide in some Asian countries[29-31].

In a study by Ryu HW *et.al*, Daphnodorin-C was shown to negatively regulate inflammatory genes expression by suppressing the activation of MAPK pathways in mouse models. Specifically, it interferes with the phosphorylation of PMK1, which is a key player in the MAPK signaling cascade responsible for various cellular responses to stress and environmental stimuli[32]. Our *in silico* screening of natural compound database NPASS against *Magnaporthe oryzae* PMK1 also identified Daphnodorin-C as a potential inhibitor of MAPK signaling cascade which is essential for

appressorium formation in the fungus and there by disease progression.

Though little literature is available on the effect of Daphnodorin A, B & C on inhibiting fungal growth, sufficient evidences are available in literature on their role in therapeutic effects. Yao *et. al*, 2011 showed that Daphnetin from *Daphne odora* when administered in collagen induced arthritis rat models, demonstrated attenuation of clinical symptoms[33]. Similarly, in a study by Takai *et.al*, 1999, Daphnodorin - A completely inhibited angiotensin-11 formation which regulates blood pressure[34].

Antifungal and insecticidal activity Studies of Daphnodorins A, B and C conducted by Namori Y *et.al*, revealed Daphnodorin-C as a good antifungal agent against *Pyricularia oryzae* (*Magnaporthe oryzae*)[35]. *In silico* experiments in this study revealed the availability of useful natural molecules of *Daphne odora*, which can be used as potential broad-spectrum biofungicides. Among the screened compounds against PMK1, Daphnodorin-C with a docking score of -10.9 kcal/mol shows that it is a potential compound for blocking the enzyme. When PMK1 which is determinant for appressorium formation was taken and docked with Daphnodorin-C it bound to the functional residues of PMK1 (VAL 30, VAL 38, GLU 32, LYS 53, ALA 34, TYR 35). The protein-ligand interactions targeted the functional residues that constituted the active sites and were in a good docking pose with least binding energy. Our *in silico* study corroborates previous published data.

The *in silico* analysis of PMK1 in complex with Daphnodorin-C has provided promising insights into the potential of this compound as an antifungal agent targeting the Mitogen-activated protein kinase (PMK1). The docking simulation revealed that Daphnodorin-C has a strong binding affinity for PMK1, with good interactions involving active site amino acid residues. This binding affinity, combined with favorable thermodynamic properties, suggests that Daphnodorin-C could effectively inhibit the activity of PMK1, which plays a crucial role in appressorium formation, which is a key process in fungal pathogenesis.

By inhibiting PMK1, Daphnodorin-C may affect the expression of genes involved in cell wall integrity and creation of turgor pressure in the appresorium. This leads to altered cellular processes such as growth, differentiation and virulence factor production in fungi[36,37]. In various fungal pathogens, including *Candida albicans* and *Magnaporthe oryzae*, disruption of PMK1 signaling has been associated with decreased virulence there by the spread of the disease. For instance, mutants lacking functional PMK1 exhibit reduced ability to infect host plants or cause disease in animal models[38,39]. PMK1 is crucial for maintaining cell wall integrity during stress conditions. Inhibition can lead to compromised cell wall structure, making fungi more susceptible to antifungal agents and less capable of withstanding host plant HR responses[40].

In addition the molecular dynamics simulation results supported the stability of the PMK1-Daphnodorin-C complex, indicating the ligand's consistent binding with minimal fluctuations throughout the simulation time. These findings underscore the potential of Daphnodorin-C as a lead compound or a precursor for the development of biofungicides targeting PMK1. The toxicity analysis stated that Daphnodorin-C has a balanced safety profile, showing moderate *in silico* toxicity, regarding hepatotoxicity or skin sensitization. Its absorption and excretion characteristics suggest a favorable pharmacokinetic profile, even though experimental testing is needed to confirm its real-world efficacy and safety. Combination of strong binding affinity, stability in molecular dynamics simulations, promising toxicity and safety profiles, makes Daphnodorin-C, a potential candidate for further experimental validation as a fungicide targeting PMK1. Further bioassays and *in vitro* studies are needed to confirm its antifungal activity and explore its potential in agricultural and therapeutic applications.

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Conflict of interest

All authors declare no conflicts of interest.

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