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Molecular docking of potential antifungal compounds from *Ulva fasciata*methanolic extract against *Pseudopestalotiopsistheae*

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ABSTRACT

Plant diseases caused by fungal pathogens significantly threaten global food security, accounting for nearly 40% of annual crop losses and incurring over US$220 billion in management costs worldwide. Among these, Pseudopestalotiopsistheae has emerged as notable phytopathogen in Sri Lanka, causing chlorosis in Solanum melongena. Its virulence is largely attributed to the secretion of pectinase enzymes, which degrade plant cell walls and facilitate host colonization. Excessive use of synthetic fungicides to manage such pathogens has led to environmental degradation, health risks, and the emergence of fungicide-resistant strains. Consequently, there is a growing interest in eco-friendly alternatives such as natural products derived from marine organisms. Marine macroalgae, particularly Ulva fasciata, commonly found in Thalpe reef, are known to produce a wide range of bioactive secondary metabolites with antifungal potential. In a previous study, methanolic extract of U. fasciata revealed numerous bioactive compounds with potential antifungal activity. The present studyaimed to evaluate the inhibitory potential of these compounds against the pectinase enzyme of P. theae using molecular docking, a powerful in silico approach for predicting interactions between small molecules and target proteins. The findings are expected to contribute to the development of sustainable, eco-friendly strategies for managing plant diseases, offering a cost-effective alternative to synthetic fungicides.This study highlights the potential of marine bioresources and computational tools in the discovery of novel antifungal agents targeting emerging phytopathogens.

Key words-Antifungal compounds, *Ulva fasciata*, Molecular docking, *Pseudopestalotiopsistheae*

INTRODUCTION

Approximately 40% of global crop production is lost each year due to attacks by pests and pathogens, including numerous bacterial and fungal species. To combat these plant diseases, more than US$ 220 billion is spent annually worldwide (FAO, 2022). Among emerging fungal pathogens, *Pseudopestalotiopsistheae*ishas been identified as a significant threat in Sri Lanka, causing chlorosis in *Solanum melongena* (Koshila *et al*., 2023). Its virulence is primarily attributed to secretion of extracellular pectinase enzymes which degrade plant cell walls and facilitate host colonization(Sopalun&Iamtham, 2020). Pectinases are a group of enzymes that hydrolyze glycosidic linkages in pectic polymers and are functionally categorized into polygalacturonases, pectin esterases, pectin lyases and pectate lyase (Arya*et al*., 2022). *Pseudopestalotiopsis*, *Neopestalotiopsis,* and *Pestalotiopsis* are closely related genera within the family Amphisphaeriaceaeand are known to cause various plant diseases, including cankers, shoot dieback, leaf spots, blights, severe chlorosis, and fruit

rot(Maharachchikumbura, 2014;Sane *et al*., 2019). Although chemical fungicides are widely used to control fungal infections,their excessive usage leads to serious environmental consequences, including contamination of aquatic ecosystems, residue accumulation in crops, and the emergence of resistant fungal strains. Moreover, fungicides pose risks to non-target organisms and human health(Goswami *et al*., 2018).

Biocontrol has been explored as a natural and sustainable alternative to chemical fungicides for managing various fungal infections in agriculture (Bubici*et al*., 2019). It involves mechanisms such as competition for space and nutrients, production of antifungal compounds and secondary metabolites (Rashad & Moussa, 2020), and the biological triggering of plant resistance (Hermosa *et al*., 2013). Plants, animals, and marine organisms are sources of natural products with inherent fungicidal activity (Dong *et al*., 2020). Marine macroalgae (seaweeds) are multicellular, eukaryotic and photosynthetic organisms known to be rich in bioactive compounds (Makkar *et al*., 2016).*Ulva fasciata*, a common macroalgae in Thalpe reef of Sri Lanka, showed potent antifungal activity against *P. theae*in a previous study through its methanolic extract (Rodrigo *et al*., 2025).Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the extract revealed several potential antifungal compounds, includingPhenylephrine, Palmitic acid, 17-Octadecenal, 4-Hydroxy-2-butanone, Heptadecene and 3-Methoxyamphetamine. However, the specific mechanism by which these compounds inhibit the fungal activityremain unclear.

Molecular docking has become a valuable computational technique for exploring the therapeutic potential of natural products. This method simulates the interactions between bioactive compounds and target proteins, predicting binding affinity and interaction modes. By virtually testing thousands of molecules, molecular docking enables the identification of promising compounds efficiently, and cost-effectively, significantly reducing the need for extensive laboratory screening (Agu *et al*., 2023). In antifungal research, docking is particularly useful for identifying inhibitors of fungal enzymes or proteins that contribute to pathogenicity. It provides insights into how candidate molecules interact with target sites at the atomic level, assessing the strength and stability of these interactions (Hendra *et al*., 2024). Hence, the present study aimed to employ molecular docking techniques to investigate the binding interactions between the most potent bioactive compounds from the methanolic extract of *U. fasciata*and the extracellular enzymes of *P. theae*, with the objective of inhibiting their enzymatic activity. Though the fungus *P. theae* secretes pectinase as an extracellular enzyme to maintain its pathogenicity, the amino acid sequences or 3D structures of pectinase enzymes from *P. theae*are not currently available in databases. Therefore, the polygalacturonase sequence from *Pestalotiopsis* sp. NC0098 (KAI0138346.1) was used to construct a homology model for subsequent analysis as it is the only available related amino acid sequence in the databases.

METHODOLOGY

Homology modeling of polygalacturonase enzyme of *Pestalotiopsis* sp.

Polygalacturonase enzyme of *Pestalotiopsis* sp. NC0098 (KAI0138346.1) was used for generating the homology model as amino acid sequences or 3D structures of pectinases of the fungus *P. theae*were not available inthe NCBI GenBank protein database.

Polygalacturonaseamino acid sequence was searched against the Protein Data Bank (PDB) using the NCBI Protein BLAST tool to identify suitable homologous templates. Four template structures with

sequence identities ranging from 54.87% to 55.46% were retrieved. Multiple sequence alignment was performed using the CLUSTALW online tool and homology modeling was carried out using MODELLER software (version 10.1).From the generated models, the one with the lowest DOPE (Discrete Optimized Protein Energy) score was selected for further analysis, as lower DOPE scores indicate higher model reliability (Selvam *et al*., 2017). The selected model was further refined in MODELLER, and energy minimization was performed using the GROMOS simulation package within Swiss-PdbViewer. Model validation was conducted using several structure assessment tools: PROCHECK, Verify3D, and ERRAT to assess stereochemical quality and 3D structure compatibility. Additionally, PROSA was used to calculate the Z-score, and the QMEAN score was evaluated using its corresponding web server to assess the overall quality and stability of the predicted structure (Selvam *et al*., 2017).

Active compound identification in the *U. fasciata*– methanolicextract

Potential antifungal compounds present in *U. fasciata*-methanolic extract were identified by GC-MS analysis as described by Kamal *et al*. (2011) in our previous study (Rodrigo *et al*., 2025).

Molecular docking

Molecular docking analysis was carried out using the AutoDock Vina software (Version 1.1.2). The homology-modeled polygalacturonase protein served as the receptor, and the receptor was prepared using Auto Dock Tools software (Version 1.5.7). The molecule was checked for adding polar H molecules and missing amino acid residues. Kollman charges were added to the molecule by equally distributing the charge across the protein surface (Phosrithong & Ungwitayatorn, 2010).

Ligand structures were based on the chemical compounds previously identified through GC-MS analysis (Rodrigo *et al*., 2025). Structures of the selected chemical molecules were obtained from the PubChem database, and energy was minimized using AVOGADRO software (Version 1.2.0). The minimized structures were then converted into a Protein Data Bank file format (pdb) using Open Babel software (Version 3.1.1). Potential ligand-binding pockets on the receptor were identified using the DoGSiteScorertool of the ProteinsPlus server. The binding pocket with the highest drug score value was selected for docking the ligands (Selvam *et al*., 2017). Nine independent docking runs were carried out for each ligand and the best binding mode with the lowest (most negative) binding free energy was selected as the best conformation (Phosrithong & Ungwitayatorn, 2010).

RESULTS AND DISCUSSION

Homology modeling of the Polygalacturonase enzyme

The extracellular enzymes are the pathogenicity determinant factors in many plant pathogens as they facilitate host invasion by degrading plant cell wall components. Enzymes are proteins that catalyze chemical reactions in living organisms, and their activity can be inhibited by certain bioactive compounds. Marine algae are known to produce diverse secondary metabolites capable of interferingwith such enzymes present in the plant pathogenic fungi and lead to the inhibition of their activity (Agu *et al*., 2023).

In this study, a homology model of the polygalacturonase enzyme was generated with 4 similar crystal structures available in the protein data bank using the MODELLER software (Figure 1). Then the loops of the structures were refined, and energy was minimized. The best model was evaluated using online servers of PROCHECK, Verfiy3D, ERRAT, PROSA, and QMEAN.

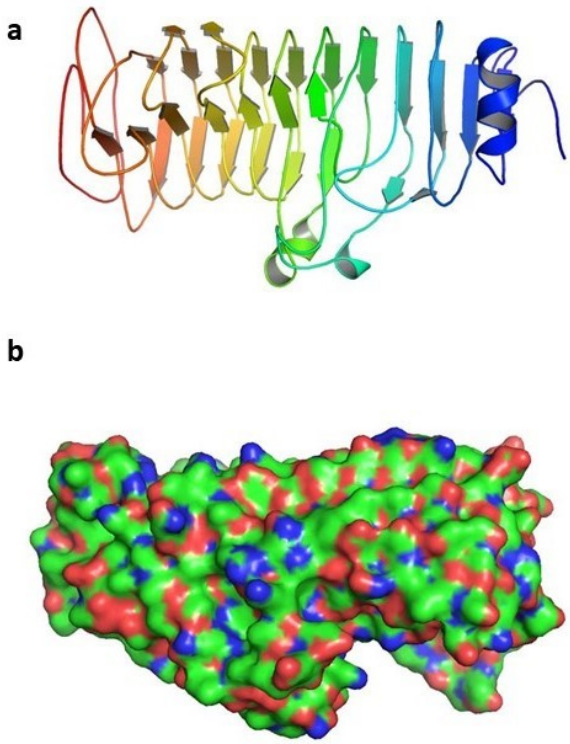


Figure 1. Homology model of polygalacturonase enzyme of *Pestalotiopsis* sp. (a) cartoon diagram (b) surface view diagram of the energy-minimized protein model

Each tool assesses different aspects of protein structure quality. PROCHECK evaluates the stereochemical quality of a protein structure including parameters like bond lengths, bond angles and planarity using Ramachandran plot analysis (Figure 2) (Wlodawer, 2017). A high percentage of residues in the most favored regions is indicative of a well-refined model. Values above 90% are considered excellent, while those exceeding 80% are generally acceptable. In this study, PROCHECK analysis revealed that 84.4% of residues (Table 1) were located in the most favoured regions of the Ramachandran plot, which falls within the acceptable range and is comparable to previous models developed for *Aspergillus niger*enzymes (Gundampati*et al*., 2012).This suggests that the overall stereochemical quality of the model is acceptable. Furthermore, no residues were observed in the generally allowed or disallowed regions (0.0%), proving the reliability of the model. The additional allowed regions (%) ideally range between 1–15%, and this model exhibited 15.6% (Table 1), which, although at the upper threshold, still falls within the acceptable range. This value is slightly higher than the percentage reported in the additionally allowed regions for *A. niger* (Gundampati*et al*., 2012). However, the overall results support the structural validity of the predicted model.

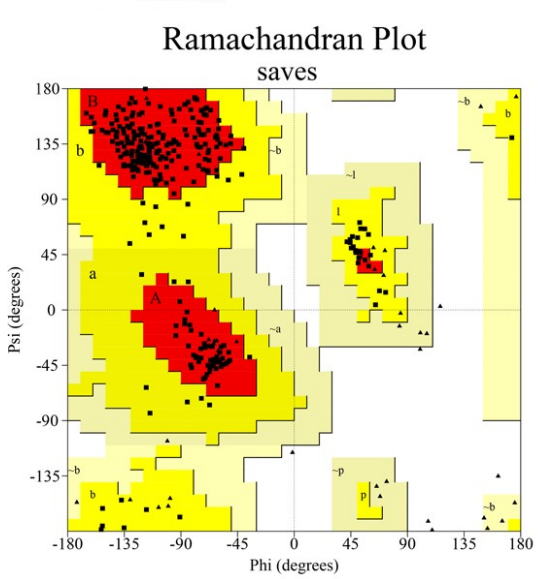


Figure 2. Ramachandran plot of the model

Table 1. Model evaluation results of PROCHECK, Verfiy3D, and ERRAT

Program	PROCHECK				Verfiy3D	ERRAT
	Most favored regions	Additional allowed regions	Generally allowed regions	Disallowed regions	3D-ID Score	Quality Factor
Value	84.4%	15.6%	0.0%	0.0%	81.07%	76.03%

Table 2. Model evaluation results of QMEAN and PROSA

Program	QMEAN	PROSA
	QMEAN4 Value	Z-Score
Value	-0.46	-6.49

Verfiy3D assesses the compatibility of the 3D model with its own amino acid sequence by assigning a 3D environment score to each residue and compares it with known preferences based on experimentally determined structures (Eisenberg *et al*., 1997).A model is generally considered reliable if a 3D-1D scoreis more than 80%.In this study, Verfy3D analysis showed 81.07% of the residues had an acceptable 3D-1D score (Table 1), indicating that the residue environments are biochemically plausible and structurally consistent.

ERRAT analyzes non-bonded atomic interactions to identify statistical deviations by comparing the input protein structure to high-resolution crystallographic data, and it computes an overall error function that reflects the model's reliability. A quality factor above 90% is indicative of an excellent model, while values between 70% and 90% are generally considered acceptable. In this study, the model achieved 76.03% quality factor,suggesting that the non-bonded interactions are largely consistent with those found in experimentally validated structures. Although this value is slightly lower

than the 83.97% reported for *Trichoderma longibrachiatum* (Tamboli*et al*., 2017), it remains within the acceptable range for functional docking studies, thereby supporting the structural plausibility of the model.

QMEAN (Qualitative Model Energy Analysis) is another important tool used to assess the quality of predicted protein structures. It is a composite scoring function that evaluates local geometry (torsion angles, solvation, hydrogen bonding), long-range interactions and agreement with high-resolution structures (Benkert *et al*., 2008). The QMEAN score typically ranges from 0 to –4, with values closer to 0 indicating a high-quality model. In this study, the QMEAN score was -0.46 (Table 2), which is close to 0 and comparable to the QMEAM values reported for *Aspergillus ficuum*, where scores were -3 or higher (Chikkerur*et al*., 2018).This suggests that the modeled structure is of good quality and comparable to experimentally determined protein structures.

PROSA provides a Z-score that indicated the energy separation of the native and average of the misfolds in the units of standard deviation (Heydari-Zarnagh*et al*., 2015). If z-score falls –4 to –10 typically indicates that global structure resembles real proteins. In our study, the PROSA Z-score was –6.49 (Table 2), which falls well within this acceptable range, suggesting that the modeled structure is realistic and reliable. This is comparable to the Z-score reported in the PROSA analysis for *Trichoderma longibrachiatum*which had a Z-score of -6.78 (Tamboli*et al*., 2017).

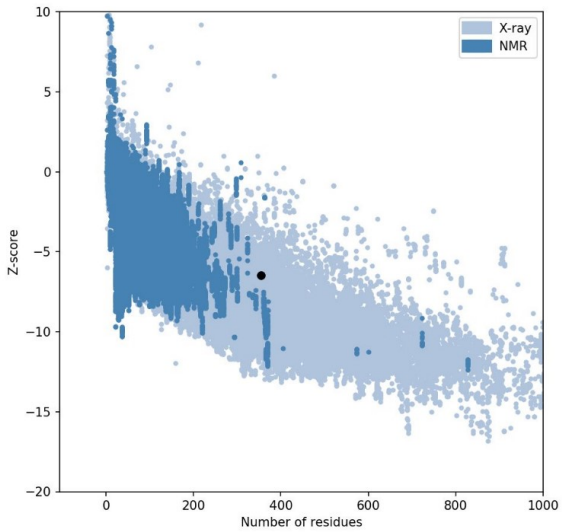


Figure 3. ProSA Z-score plot of the model. The value of Z-score is highlighted as a black dot and is in the range of native conformations

This multi-angle validation is essential to build trust in the accuracy of a predicted or experimentally determined protein model before using it in downstream applications like molecular docking, drug design, or structural biology research. The scores received for these tests indicate that the model generated was of good quality, and it has higher reliability (Selvam *et al*., 2017).

Biologically active compounds in the *U. fasciata*– methanolic extract

Nine different chemical compounds were identified in our previous study by Rodrigo *et al*. (2025) using GC-MS analysis (Table 3). Various aromatic and non-aromatic compounds were found in different abundances (Figure 3). The most abundant compounds in the extract were 4-hydroxy-2-butanone (30.75%) followed by hydroxylamine/methylamine (37.37%).

Table 3. Chemical compounds identified from of *U. fasciata*-methanolic extract

Compound	Retention time (min)	Compound ID	Molecular formula	% of total
Phenylephrine 3-Methoxyamphetamine	01.10	6041 152234	$C_9H_{13}NO_2$ $C_{10}H_{15}NO$	00.12
Hydroxylamine Methylamine	01.14	787 6329	H_3NO CH_5N	37.37
4-Hydroxy-2-butanone	01.15	111509	$C_4H_8O_2$	40.75
17-Octadecenal	02.33	41922	$C_{18}H_{34}O$	01.75
Palmitic acid	16.05	985	$C_{16}H_{32}O_2$	00.57
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,(1alpha,2beta,5alpha)	16.80	12314300	$C_{10}H_{18}$	0.57
1,2-Benzisothiazol-3-amine	25.678	89966	$C_7H_6N_2S$	0.03

When considering previously published data, variations in chemical composition have been reported even among extracts from the same species (Abbassy*et al*., 2014; Shobier*et al*., 2016). These differences are likely due to variations in secondary metabolites profiles, which can be influenced by factors such as geographical locations, environmental conditions, and the development stage of the algae.

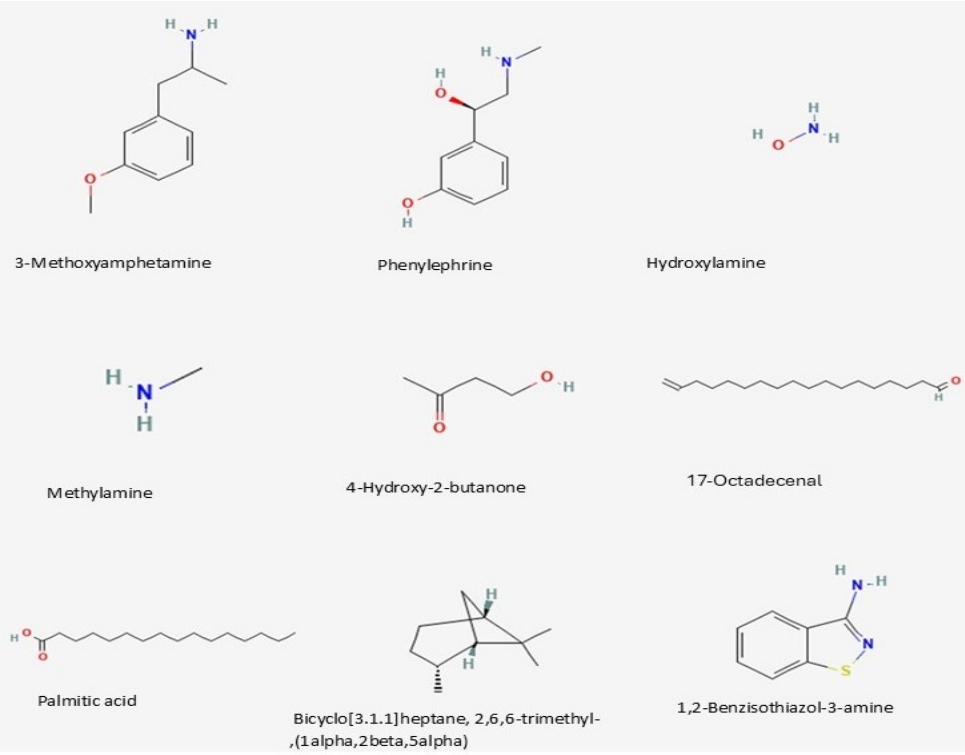


Figure 4. Structures of the studied bioactive compounds in *U. fasciata*-methanolic extract

Palmitic acid (RT=18.056 min) was detected in this study, and it has commonly been reported in previous studies of *Ulva*-methanol extract (Abbassy*et al*., 2014; Barot *et al*., 2016; Shobier*et al*., 2016). The exact mechanisms by which antifungal compounds derived from macro algae effect fungal growth inhibition are still not fully understood. But several hypotheses have been proposed. Compounds found in algal extracts may exert antifungal effects through multiple mechanisms, including disruption of cell wall and membrane integrity, interference withkey intracellular components like the nucleus and mitochondria, inhibition of protein synthesis and enzymatic functions and impairment of the mitochondrial respiratory chain. These actions collectively destabilize cellular homeostasis, ultimately reducing the fungal cell's viability and lifespan (Lopes *et al*., 2013). Additionally, certain fatty acids from macroalgae exhibit antifungal activity by incorporating into the fungal membrane, altering its fluidity and permeability, and reorganizing its structure, which ultimately leads to cell death (Avis & Bélanger, 2001). Molecular docking can predict the binding site and the strength of the interaction between these algal chemical compounds and the fungal extracellular enzymes, which can help in understanding the mechanism of action.

Molecular docking

If the methanolic extract of*U.fasciata*- could significantly inhibit fungal activity, it is plausible that its bioactive compounds may have the ability to bind with extracellular enzymes and inhibit their function. To check this possibility, an *in-silico* analysis was carried out. Potential ligand binding sites of the target protein model were identified using the DoGSiteScorertool which predicts druggable pockets based on protein structure analysis. The best binding pocket (Figure 4) was selected based on its highest drug score value of 0.81. The volume of the pocket was 1422.87 Å 3 and the surface area was 1413.39 Å 2. It comprised 322 atoms and featured 82 H-bond acceptor sites and 31 H-bond donor sites.

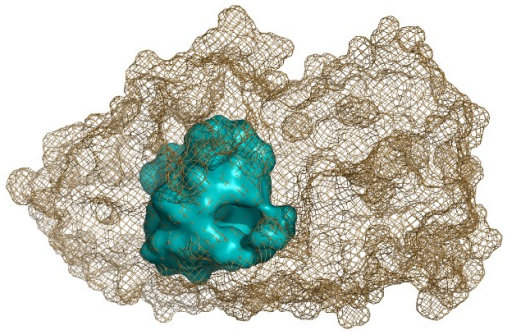


Figure 5. The best possible ligand-binding pocket of the protein model (green color area)

The 3D structures of nine compounds identified from GC-MS analysis were prepared as ligands for molecular docking. Docking simulations were performed using AutoDock Vina, which predicted the binding affinities of all nine ligands. Each ligand was docked into the selected binding pocket of the target protein model. All nine ligands successfully bound within the selected pocket, with predicted binding free energies ranging from –2.2 to –5.8 kcal/mol (Table 4).

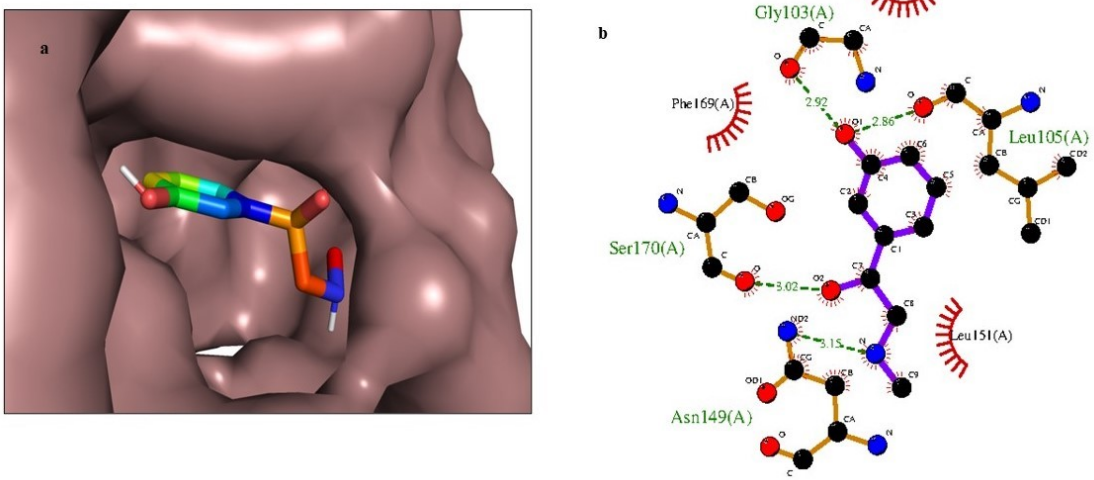
Binding free energy (kcal/mol) provides a numerical estimate of the strength of interaction between a ligand and a target protein, where more negative values indicate stronger predicted binding affinities. Generally, values below –7.0 kcal/mol indicate strong binding potential, –5.0 to –7.0 kcal/mol suggest

moderate but stable binding interactions, and –3.0 to –5.0 kcal/mol indicate relatively weaker binding (Meng *et al*., 2011).

Table 4. Best docking score of ligands with the protein model

Ligand name	Docking score of the best conformation (kcal/mol)
1,2-Benzisothiazol-3-amine	-5.0
3-Methoxyamphetamine	-5.0
4-Hydroxy-2-butanone	-3.6
17-Octadecenal	-4.4
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,(1alpha,2beta,5alpha)	-5.8
Hydroxylamine	-3.7
Methylamine	-2.2
Palmitic acid	-4.7
Phenylephrine	-5.7

The highest docking score was observed forBicyclo[3.1.1]heptane,2,6,6 trimethyl,(1alpha,2beta,5alpha) (-5.8 kcal/mol), followed by Phenylephrine (-5.7 kcal/mol), revealing stronger predicted binding affinities compared to the other ligands and suggesting a greater potential to form stable interactions with the extracellular enzyme (Selvam *et al*., 2017). Figures 5 and 6 illustrate the docking complexes and the interactions between these ligands and amino acid residue within the binding pocket. Notably, both ligands form multiple hydrogen bonds and hydrophobic interactions, which are critical for stabilizing ligand-protein complexes. These findings suggest that the compounds may inhibit enzymatic activity by occupying or altering the confirmation of the active site, thereby preventing substrate binding or catalytic function.



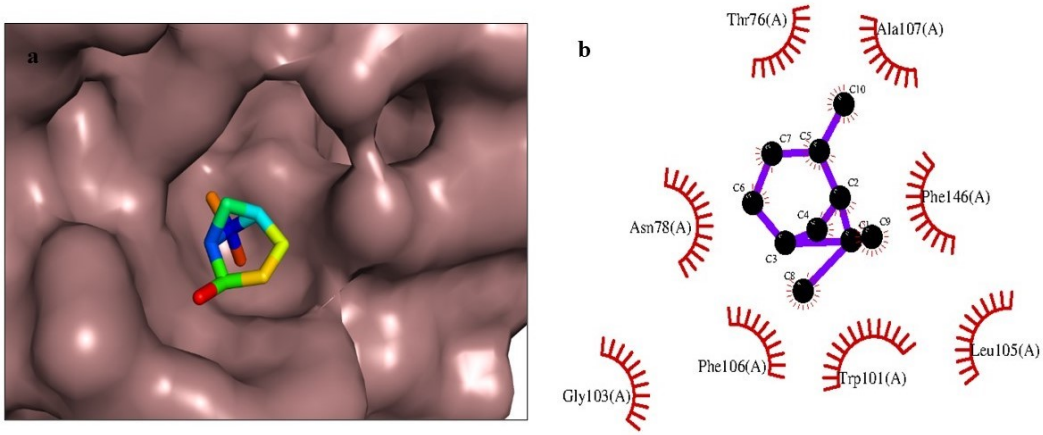


Figure 6. Docking complex and interactions of bicyclo[3.1.1]heptane,2,6,6-trimethyl-,(1alpha,2beta,5alpha) and the amino acids of the model for, (a) 3D structure (b) 2D structure

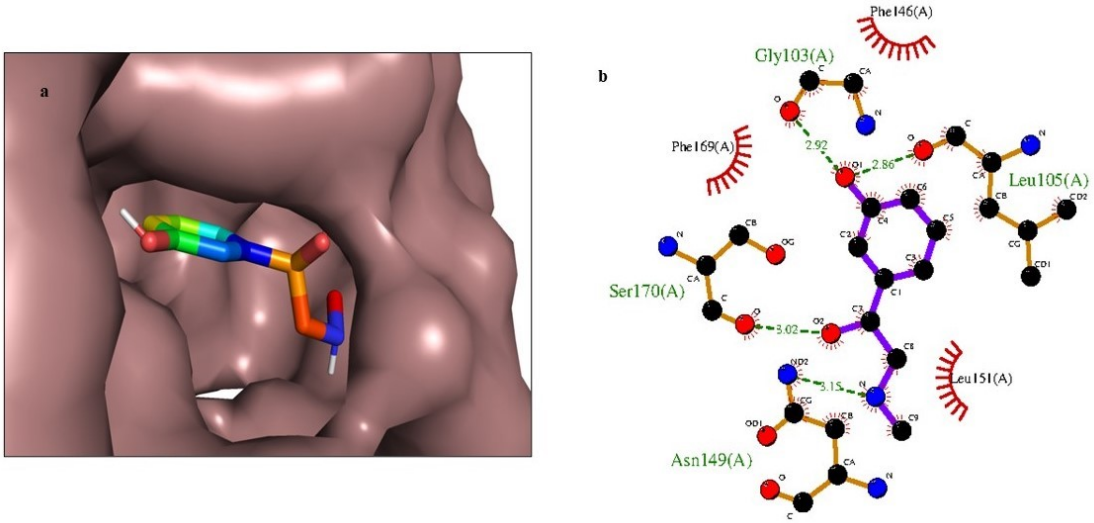


Figure 7. Docking complex and interactions of Phenylephrine and the amino acids of the model for, (a) 3D structure (b) 2D structure

Furthermore, compounds such as 1,2-Benzisothiazol-3-amine (-5.0 kcal/mol) and 3-Methoxyamphetamine (-5.0 kcal/mol) showed moderately strong binding affinities, suggesting they may contribute synergistically to the overall antifungal activity of the extract. The presence of multiple bioactive compounds with diverse binding profiles may enable the extract to target a broader range of fungal enzymes, thereby enhancing its overall efficacy. Thesefindings showed the potential of marine macroalgae-derived compounds as enzyme inhibitors, and support their promise as a sustainable source for development of novel antifungal agents.

CONCLUSION

In this study, molecular docking simulations were employed to identify potential antifungal targets of compounds present in the *U. fasciata*-methanol extract. The docked ligands demonstratednotable binding interactions with the polygalacturonase enzyme of *Pestalotiopsis*, a key pathogenic determinant. Among the tested compounds, Bicyclo[3.1.1]heptane,2,6,6-trimethyl-

,(1alpha,2beta,5alpha) (-5.8 kcal/mol) and Phenylephrine (-5.7 kcal/mol) exhibited the highest binding affinities, indicating stable and moderate interactions with the enzyme. These findings provide preliminary evidence supporting the antifungal potential of *U. fasciata* metabolites and highlight their possible applications in developing sustainable, macroalgae-derived fungicides for crop protection against fungal pathogens.

ACKNOWLEDHMENT

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