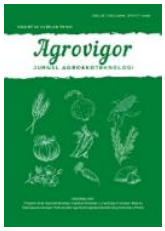
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Article

Molecular Docking-Based Identification of Betel Leaf Bioactive Compounds as Potential Cutinase Inhibitors of *Colletotrichum gloeosporioides*

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ABSTRACT

Anthracnose, caused by *Colletotrichum gloeosporioides*, is a major disease affecting horticultural crops because it can significantly reduce both the quality and quantity of harvested products. Cutinase plays a crucial role in the early stages of infection by enabling the pathogen to penetrate the plant cuticle, which serves as the first line of host defense. This study aimed to evaluate the potential of bioactive compounds from betel as inhibitors of *C. gloeosporioides* cutinase using molecular docking. A total of nine compounds were analyzed through PASS biological activity prediction, ADMET-based physicochemical and toxicity assessment, and molecular docking simulations. The PASS analysis revealed that all compounds had higher probability of activity (Pa) values than probability of inactivity (Pi) values, indicating potential antifungal activity. Among the tested compounds, β-sitosterol exhibited the highest Pa value (0.585) and the strongest binding affinity toward cutinase, with a binding energy of −7.1 kcal/mol and an inhibition constant (Ki) of 6.20 μM. These values were superior to those of the co-crystallized ligand (−4.8 kcal/mol; Ki = 301.60 μM) and the reference fungicide mancozeb (−3.2 kcal/mol; Ki = 4497.40 μM). Piperine and piperlonguminine also demonstrated strong binding affinities, with binding energies of −6.7 and −6.5 kcal/mol, respectively. The stability of the β-sitosterol–cutinase complex was primarily supported by hydrophobic and van der Waals interactions with key residues within the enzyme's active site. Based on the in-silico analysis, β-sitosterol demonstrated the greatest potential as an inhibitor candidate among the compounds tested. However, these findings are predictive and require further validation through in vitro and in vivo studies to confirm its biological activity, efficacy, and safety.

INTRODUCTION

Anthracnose is a major disease affecting various horticultural and plantation crops because it can reduce both harvest quality and quantity. One of the primary causal pathogens is *Colletotrichum gloeosporioides*, which has a broad host range and can infect plants during both pre-harvest and post-harvest stages. Infection by this pathogen is generally characterized by necrotic spots, fruit rot, and plant tissue damage, leading to economic losses (Law et al., 2025). Appressorium formation in *Colletotrichum* species is critical for successful infection because this structure enables the pathogen to recognize plant surface signals and penetrate the intact cuticle during the initial stage of host colonization (Kodama et al., 2023).

One enzyme that plays a key role in fungal pathogen penetration is cutinase (Arya & Cohen, 2022). The ability of *Colletotrichum* spp. to produce and secrete cutinase is crucial to its infection strategy, as this enzyme degrades the plant cuticle and supports the development of the pathogen's necrotrophic phase (Villafana & Rampersad, 2020). Therefore, cutinase can be considered a potential molecular target for developing antifungal compounds that inhibit infection mechanisms. Compounds capable of binding to the active site of cutinase may inhibit enzyme activity and reduce the pathogen's ability to infect plants.

The control of anthracnose has so far relied heavily on synthetic fungicides. However, intensive fungicide use has been associated with various negative impacts, including residue accumulation, environmental degradation, the emergence of resistant pathogens, and risks to human health and non-target organisms (Choudhary et al., 2018). These conditions highlight the need to explore more environmentally friendly alternative active ingredients, including plant-derived bioactive compounds as potential botanical fungicide candidates.

Betel leaves (*Piper betle* L.) are rich in bioactive compounds, such as phenols, flavonoids, and alkaloids, which contribute to various biological activities, including antimicrobial activity (George et al., 2024). These compounds have been reported to possess antimicrobial and antifungal properties (Aoki et al., 2019; Charirak et al., 2023), making betel leaves a potential source of natural active ingredients for controlling plant pathogens. Betel leaf extract also exhibits strong antifungal activity against *Sclerotium rolfsii*, completely inhibiting pathogen growth in *in vitro* assays (Hai et al., 2025). Volatile organic compounds from *Bacillus* have been reported to interact with cutinase, a key virulence-related enzyme of *C. gloeosporioides* (Putra et al., 2026). Similarly, a previous study demonstrated the antifungal potential of *Piper betle* compounds against *C. gloeosporioides* cutinase by evaluating extract-derived constituents using DFT calculations, molecular docking, and ADMET prediction (Nhung et al., 2024). The present study extends this work by screening a distinct set of betel leaf compounds, including piperine, piperlonguminine, carvacrol, estragole, triacontane, and dotriacontanoic acid, through an integrated approach combining PASS-based antifungal activity prediction, physicochemical and toxicity assessment, molecular docking, inhibition constant estimation, and comparative evaluation against both the co-crystallized ligand and the commercial fungicide mancozeb. Thus, the novelty of this study lies in expanding the chemical diversity of betel leaf compounds assessed against cutinase and integrating multiple predictive parameters to prioritise potential inhibitor candidates.

MATERIALS AND METHODS

Research methods

This study was conducted in silico to identify bioactive compounds from betel leaf as candidate inhibitors of the cutinase enzyme of *C. gloeosporioides*. The research stages included compound identification, prediction of biological activity, analysis of physicochemical properties and toxicity, and molecular docking simulations.

Compound identification and biological activity prediction

Bioactive compounds from betel leaf were obtained from the KNApSAcK database (https://www.knapsackfamily.com/knapsack_core/top.php) using the keyword "*Piper betle*". The chemical structures of the identified compounds were then retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Biological activity prediction was performed using the PASS Online web server available through the Way2Drug platform (https://way2drug.com/PASSonline/). Compounds with Pa values higher than Pi values, particularly those with activities relevant to antifungal properties, were selected for further analysis.

Analysis of Physicochemical Properties and Toxicity

The selected compounds were analyzed in silico for their physicochemical properties and toxicity using the ADMETlab 3.0 web server (https://admetlab3.scbdd.com/) to assess their suitability as potential botanical fungicide candidates. The physicochemical parameters evaluated included molecular weight (MW), LogP, LogS, topological polar surface area (TPSA), and the number of rotatable bonds (nRot). The toxicity parameters analyzed included rat oral acute toxicity, AMES toxicity, carcinogenicity, skin sensitization, eye irritation, and LC50 against *Daphnia magna* (LC50DM). Although these parameters are commonly used in pharmaceutical research, in this study they were employed as preliminary indicators of physicochemical suitability, potential human toxicity, and environmental safety relevant to the development of botanical fungicides.

Preparation of Enzyme Structure and Ligands

The three-dimensional (3D) and two-dimensional (2D) structure of the target enzyme, the *Glomerella cingulata* PETFP-cutinase complex (PDB ID: 3DEA), was obtained from the Protein Data Bank (PDB; https://www.rcsb.org/structure/3DEA). The enzyme was prepared by removing water molecules, ions, and co-crystallized ligands, followed by the addition of polar hydrogen atoms and partial charges. Binding pocket validation was performed using PrankWeb (https://prankweb.cz) on the cutinase enzyme structure that had been prepared and used as the receptor in the molecular docking process. The selected pocket was determined based on its ranking, score, probability, and alignment with the active site residues reported in the literature. The pocket center coordinates were used to evaluate the suitability of the docking grid box location. Key active-site residues, including Ser57, Ser136, and Gln137, were determined based on previous literature and binding pocket analysis of the PDB structure.

Molecular Docking Simulation

Molecular docking was performed using AutoDock Vina to evaluate the binding affinities of the selected ligands toward the target cutinase enzyme. The protein and ligand structures were prepared and converted into PDBQT format before docking. The docking grid was positioned within the enzyme's active site, encompassing the key residues Ser57, Ser136, and Gln137. The docking region was defined using a grid box of 20 × 20 × 20 Å, centered at x = 9.175, y = 24.317, and z = 34.418. The exhaustiveness parameter was set to 16 to improve ligand conformational sampling, while the maximum number of generated poses (num_modes) was set to nine. The pose with the lowest binding energy was selected as the most favorable conformation for subsequent analysis of ligand–residue interactions within the protein-binding site. The docking protocol was validated by redocking the co-crystallized ligand into the active site of the cutinase enzyme using the same docking parameters. The redocked pose was superimposed on the native crystallographic conformation, resulting in an RMSD value of 1.475 Å. Because this value was below the generally accepted threshold of 2.0 Å, the docking protocol was considered valid and suitable for subsequent ligand screening. Identical docking settings were applied to all ligands to maintain consistency and comparability among the results. The pose with the lowest binding energy was chosen as the most favorable binding conformation for subsequent analysis (Flores-Castañón et al. 2022).

Analysis of Binding Energy and Molecular Interactions

Ligand affinity for the target enzyme was evaluated based on the binding energy (kcal/mol) obtained from molecular docking simulations (Vaidyanathan et al., 2023). The inhibition constant (Ki) was then calculated based on the binding energy to predict the inhibitory potential of each compound against the target enzyme. Interactions between the ligand and the protein were analyzed by examining the presence of hydrogen bonds, hydrophobic interactions, π–alkyl interactions, and van der Waals forces, particularly those involving key residues at the active site, namely Ser57, Ser136, and Gln137. The relationship between binding energy values and the number and type of molecular interactions is discussed descriptively. The inhibition constant (Ki) is calculated from the binding energy (ΔG) using the following thermodynamic equation (Pandey & Verma, 2022):

$$K_i = e^{\frac{\Delta G}{RT}} \quad (1)$$

K_i= inhibition constant (M)
ΔG= binding energy (kcal/mol)
R= gas constant (1,987 × 10^{-3} kcal·mol^{-1}·K^{-1})
T= absolute temperature (K), typically 298 K

Data Analysis

The data were analyzed descriptively based on Pa and Pi values, physicochemical parameters, toxicity, binding affinity, and ligand–protein interaction patterns. Interaction visualization was performed using Discovery Studio Visualizer and PyMOL. The best compound was determined based on a combination of molecular docking result and the ability to interact with key residues in the active site of the cutinase enzyme.

RESULTS AND DISCUSSION

Molecular docking-based prediction of antifungal activity and chemical class of bioactive compounds in betel leaf

The biological activity prediction results indicated that all bioactive compounds from betel leaf had higher Pa values than Pi values, suggesting potential antifungal activity (Table 1). A Pa value > 0.7 indicates a high probability of biological activity in experimental assays, 0.5 < Pa < 0.7 indicates moderate probability, while Pa < 0.5 indicates low probability but possible potential as a new chemical entity if experimentally confirmed (Lagunin et al., 2000). Several compounds with Pa values below 0.3 were retained because their Pa values remained higher than their corresponding Pi values. In addition, these compounds have been reported as bioactive constituents of betel leaves or as having the potential to interact with active-site residues. Therefore, they were included as exploratory candidates, although further validation is still required. β-Sitosterol exhibited the highest Pa value (0.585), with a Pi value of 0.020, making it the compound with the greatest predicted antifungal potential. β-Sitosterol is a phytosterol involved in cell membrane stabilization; therefore, its antimicrobial potential may be associated with its ability to affect microbial membrane integrity and function (Bin Sayeed et al., 2016). Furthermore, β-sitosterol has been reported to inhibit *Cephalosporium maydis* growth *in vitro*, suppress late wilt disease incidence in maize, and enhance plant defense enzyme activity and crop yield under greenhouse and field conditions (Elshahawy & Abd El-Wahed, 2022).

Other compounds, including eugenol, chavicol, carvacrol, estragole, dotriacontanoic acid, and triacontane, also exhibited antifungal potential, with Pa values ranging from 0.377 to 0.460. Phenolic compounds, such as eugenol and carvacrol, are known to possess broad-spectrum antimicrobial activity through mechanisms involving cell membrane damage, increased membrane permeability, leakage of intracellular components, and disruption of key enzymatic activities in pathogens (L. Chen et al., 2026; Marchese et al., 2017). In contrast, piperine and piperlonguminine showed lower Pa values of 0.176 and 0.213, respectively. Based on these results, betel leaf bioactive compounds with Pa > Pi were selected as primary candidates for further molecular docking analysis against the cutinase enzyme of *C. gloeosporioides*. Although piperine and piperlonguminine showed relatively low Pa values, both were retained in the subsequent analysis because their Pa values remained higher than their Pi values, indicating potential relevant biological activity. Overall, these prediction results provide a basis for further evaluation through ADMET analysis and molecular docking to confirm the suitability of these compounds as candidate cutinase inhibitors against *C. gloeosporioides.*

Molecular docking prediction of ADMET properties of bioactive compounds from betel leaf

Table 2 shows that the bioactive compounds in betel leaves possess diverse physicochemical properties. Low-molecular-weight compounds, such as chavicol, carvacrol, eugenol, and estragole, have molecular weights ranging from 134.07 to 164.08, moderate LogP values, and low nRot values. These characteristics generally indicate small molecular size, low molecular flexibility, and the potential for more stable interactions with target proteins (Darlami & Sharma, 2024). The LogS values of these compounds are also relatively higher than those of the other compounds,

although they remain negative, indicating limited water solubility but not as low as that of long-chain hydrocarbons. Piperine and piperlonguminine have moderate molecular weights, with higher TPSA values of 38.77 and 47.56, respectively, suggesting their potential to form polar interactions, such as hydrogen bonds, with target amino acid residues. In contrast, triacontane, dotriacontanoic acid, and β-sitosterol exhibit high LogP values and very low LogS values, indicating high hydrophobicity and low water solubility. This low water solubility is attributed to the predominance of nonpolar hydrocarbon chains, which confer high hydrophobicity and limit interactions with water molecules (Paaver et al., 2016). Triacontane and dotriacontanoic acid also have very high nRot values of 27 and 30, respectively, indicating that their molecular structures are highly flexible. Physicochemical properties and drug-likeness parameters are key components in the initial evaluation of a compound, as they provide valuable insights into its biological activity, pharmacokinetic profile, and potential for further development as a bioactive agent (Ahmad et al., 2023). Overall, chavicol, carvacrol, eugenol, estragole, piperine, and piperlonguminine exhibit physicochemical profiles that are more suitable for further analysis, whereas β-sitosterol, triacontane, and dotriacontanoic acid require careful consideration due to their hydrophobic nature and low solubility.

Table 1. Profile of Bioactive Compounds from Betel Leaf and Their Predicted Antifungal Activity Based on Pa and Pi Values

Compounds	Class	Molecular formula	CAS	Pa	Pi	Activity	Origin
Chavicol	Phenylpropanoid	C9H10O	501-92-8	0.449	0.039	Antifungal	*Piper betle*
Triacontane	Alkane hydrocarbons	C30H62	630-05-7	0.377	0.055	Antifungal	*Piper betle*
Dotriacontanoic acid	Fatty acids	C32H64O2	3625-52-3	0.407	0.048	Antifungal	*Piper betle*
(-)-beta-Sitosterol	Phytosterol	C29H50O	83-46-5	0.585	0.020	Antifungal	*Piper betle*
Piperine	Amide alkaloids	C17H19NO3	94-62-2	0.176	0.155	Antifungal	*Piper betle*
Piperlonguminine	Amide alkaloids	C16H19NO3	5950-12-9	0.213	0.129	Antifungal	*Piper betle*
Carvacrol	Phenolic monoterpenoids	C10H14O	499-75-2	0.449	0.039	Antifungal	*Piper betle*
Eugenol	Phenylpropanoid	C10H12O2	97-53-0	0.460	0.037	Antifungal	*Piper betle*
Estragol	Phenylpropanoid	C10H12O	140-67-0	0.425	0.045	Antifungal	*Piper betle*

Table 2. Molecular docking Physicochemical Properties of Bioactive Compounds from Betel Leaf

Compounds	MW	LogP	LogS	TPSA	nRot
Chavicol	134.07	2.532	-1.328	20.23	2.0
Triacontane	422.49	10.353	-10.083	0.0	27.0
Dotriacontanoic acid	480.49	9.819	-8.826	37.3	30.0
(-)-beta-Sitosterol	414.39	8.004	-7.221	20.23	6.0
Piperine	285.14	2.509	-3.905	38.77	4.0
Piperlonguminine	273.14	3.218	-4.096	47.56	6.0
Carvacrol	150.1	3.218	-2.115	20.23	1.0
Eugenol	164.08	2.321	-1.891	29.46	3.0
Estragol	148.09	2.895	-2.788	9.23	3.0

Table 3 shows the predicted toxicity and ecotoxicity of bioactive compounds from the betel plant. Based on the rat oral acute toxicity parameter, the highest value was observed for dotriacontanoic acid at 0.680, followed by carvacrol at 0.417 and eugenol at 0.346. In contrast, the lowest values were found for β-sitosterol at 0.111 and triacontane at 0.142. In the AMES test, piperine and piperlongumine showed the highest values, at 0.761 and 0.724, respectively, indicating that both compounds have a greater predicted tendency for mutagenicity than the other compounds. The carcinogenicity parameter also showed the highest value for piperine at 0.732, followed by piperlongumine at 0.697 and β-sitosterol at 0.688. Meanwhile, triacontane and dotriacontanoic acid had the lowest AMES and carcinogenicity values, indicating a lower predicted tendency for mutagenic and carcinogenic risks. For the skin sensitization and eye irritation parameters, most compounds showed high values, particularly triacontane, dotriacontanoic acid, chavicol, eugenol, and estragole, indicating their potential to cause skin sensitization and eye irritation. The highest LC50DM values were observed for triacontane (6.875) and dotriacontanoic acid (6.569), whereas the lowest value was observed for chavicol (4.272). Toxicity prediction is a critical step in the initial evaluation of bioactive compounds because it provides information on their potential adverse effects on humans, animals, plants, and the environment (Raies & Bajic, 2016). Overall, these results indicate that each compound has a distinct toxicity profile, with piperine and piperlongumine standing out in terms of AMES and carcinogenicity risks, while most other compounds require further attention regarding their potential for skin sensitization and eye irritation.

Table 3. Molecular docking Prediction of Toxicity and Ecotoxicity of Bioactive Compounds from Betel Leaf

Compounds	Rat Oral Acute Toxicity	AMES	Carcinogenicity	Skin Sensitization	Eye Irritation	LC50DM
Chavicol	0.309	0.391	0.566	0.963	0.996	4.272
Triacontane	0.142	0.008	0.141	0.999	0.998	6.875
Dotriacontanoic acid	0.068	0.004	0.127	0.998	0.998	6.569
(-)-beta-Sitosterol	0.111	0.139	0.688	0.99	0.956	5.144
Piperine	0.272	0.761	0.732	0.607	0.633	5.227
Piperlonguminine	0.187	0.724	0.697	0.858	0.941	5.486
Carvacrol	0.417	0.398	0.606	0.717	0.996	4.578
Eugenol	0.346	0.468	0.582	0.93	0.993	4.542
Estragol	0.281	0.502	0.644	0.881	0.992	4.546

Molecular docking analysis of bioactive compounds against the cutinase enzyme

The pathogenic fungus *C. gloeosporioides* is known as one of the causal agents of anthracnose, which frequently affects horticultural crops, particularly fruits (Guo et al., 2022). The infection process is highly dependent on the pathogen's ability to penetrate the plant cuticle, the initial protective layer of host tissue. This penetration is facilitated by cutinase, an extracellular enzyme capable of degrading cutin and playing a crucial role in the early stages of infection and disease development (Villafana & Rampersad, 2020). The role of cutinase as a virulence factor has also been reported in *Colletotrichum truncatum*, particularly through the involvement of the CtCut1 gene in infection and pathogenicity (Auyong et al., 2015). Thus, cutinase can be considered a potential target in enzyme-inhibition-based anthracnose control strategies. In this *in silico* study, molecular docking was used to assess the ability of bioactive compounds to inhibit cutinase by analyzing binding affinity and interaction patterns with the key residues Ser57, Ser136, and Gln137 at the active site of the *C. gloeosporioides* enzyme (Nyon et al., 2009). Compounds with lower binding energies and smaller Ki values tend to form more stable interactions with these residues, thereby potentially inhibiting the catalytic activity of cutinase (Copeland, 2005).

Based on the PrankWeb analysis, one main binding pocket was identified and ranked first, with a score of 6.60, a probability of 0.364, and 16 constituent residues with an average conservation score of 2.437. Visually, the predicted pocket was located within the main cavity on the surface of the cutinase structure, as indicated by the red region in Figure 1. These findings support the selected grid box location and demonstrate that the docking site was determined not only from active-site residues reported in the literature but also from structure-based binding-pocket prediction. However, the predicted probability suggests a moderate level of confidence; therefore, experimental validation is still required to confirm the functional role of this pocket in ligand binding and the inhibition of cutinase activity.

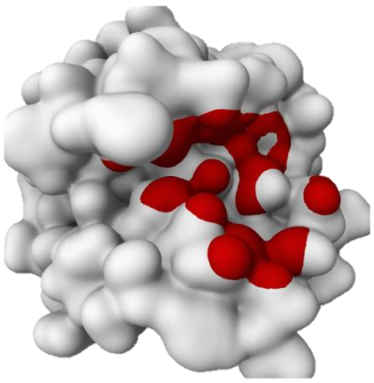


Figure 1. Prediction of the binding pocket of *C. gloeosporioides* cutinase using PrankWeb.

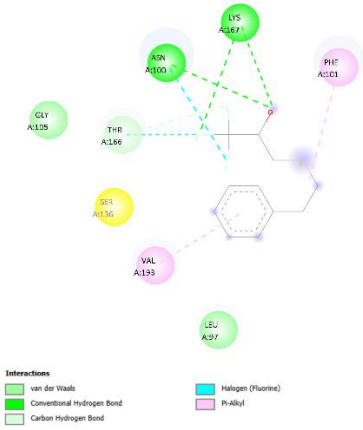
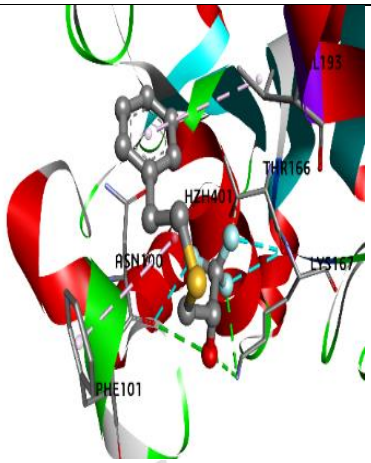
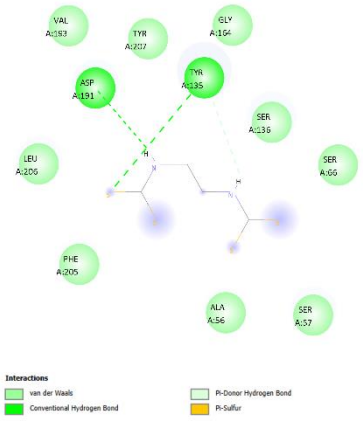
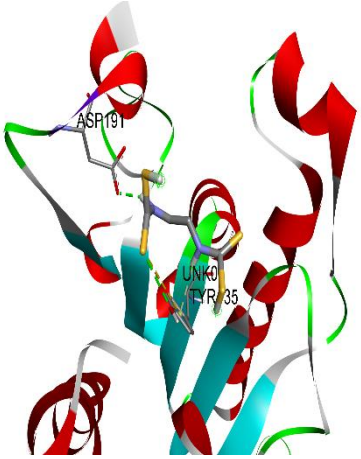
Validation of the docking protocol was performed by redocking the co-crystallized ligand into the active site of the target protein. Comparison of the docking pose with the native ligand conformation yielded a root mean square deviation (RMSD) value of 1.475 Å. This value indicates that the docking procedure is sufficiently valid and reliable, as it falls below the generally accepted threshold of 2.0 Å (Amrulloh et al., 2023). Furthermore, the molecular docking results showed that the analyzed bioactive compounds exhibited varying binding affinities toward the cutinase enzyme. These differences were reflected in the binding energy and inhibition constant (Ki) values, as presented in Table 5. Based on the 2D and 3D interaction visualizations, (-)-β-sitosterol exhibited the best inhibitory potential against the target protein (Table 4). Based on the 2D and 3D interaction visualizations, (-)-β-sitosterol exhibited the strongest inhibitory potential against the target protein (Table 4). This was indicated by its lowest binding energy value of −7.1 kcal/mol and smallest inhibition constant (Ki) value of 6.20 μM (Table 5). These values were more favorable than those of the co-crystal ligand (−4.8 kcal/mol; Ki = 301.60 μM) and the reference fungicide mancozeb (−3.2 kcal/mol; Ki = 4497.40 μM). In molecular docking studies, lower binding energy generally indicates stronger ligand–receptor affinity and greater complex stability, while a smaller Ki value suggests higher inhibitory potential (Du et al., 2016). In addition, the Ki value can be derived from the binding free energy through the thermodynamic relationship between ΔG and the inhibition constant and can therefore be used as a quantitative indicator of a ligand's inhibitory potential against a target protein (Ortiz et al., 2019). Although (-)-β-sitosterol does not form hydrogen bonds, the 2D and 3D interaction visualizations indicate that the stability of the ligand–protein complex is primarily maintained by hydrophobic and van der Waals interactions with amino acid residues at the active site, including Ser57 and Ser136. Noncovalent interactions, such as van der Waals forces, hydrophobic interactions, and hydrogen bonds, are known to play crucial roles in stabilizing protein–ligand complexes (Du et al., 2016). Previous docking studies have also shown that β-sitosterol predominantly interacts with target proteins through hydrophobic contacts, which contribute significantly to the stability of the ligand–protein complex (Anggreini et al., 2023).

Other compounds that also exhibited strong affinity were piperine and piperlongumine, with binding energies of -6.7 and -6.5 kcal/mol, respectively, and Ki values of 12.19 and 17.08 μM, respectively. Although both compounds exhibited low binding energies, only piperine interacted with key residues through conventional hydrogen bonds, specifically Ser57 and Ser136. Piperlongumine formed a conventional hydrogen bond with Lys167, which was not

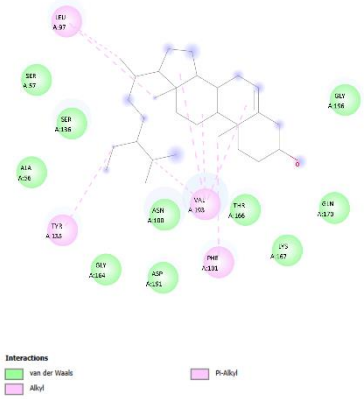
considered a key residue. However, its interaction with key residues Ser57 and Ser136 through van der Waals forces may still contribute to binding stability. The 2D and 3D interaction diagrams indicated that these two compounds were stabilized by hydrophobic interactions within relatively stable binding sites and adopted orientations that supported interactions with the protein's active residues. Piperine forms conventional hydrogen bonds with the key residues Ser57 and Ser136, which may contribute to the stability of the protein–ligand complex. Hydrogen bonding is recognized as one of the primary factors governing binding affinity and molecular recognition between proteins and ligands (D. Chen et al., 2016).s

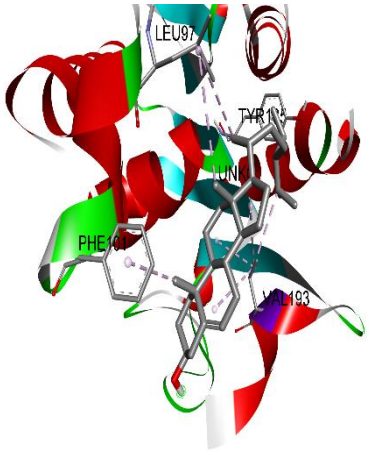
Meanwhile, eugenol exhibited moderate affinity toward the target protein, with a binding energy of -5.1 kcal/mol and a Ki value of 181.72 μM. This compound formed a hydrogen bond and hydrophobic interactions. Carvacrol, chavicol, estragole, and triacontane exhibited lower affinity, as indicated by their binding energies ranging from -4.5 to -4.1 kcal/mol and their relatively higher Ki values. The interactions of these compounds were predominantly governed by van der Waals and alkyl/π-alkyl interactions; however, the number and strength of these interactions were lower than those observed for (-)-β-sitosterol, piperine, and piperlongumine. Dotriacontanoic acid exhibited the weakest affinity, with a binding energy of only -0.8 kcal/mol and a very high Ki value of 258,964.63 μM, indicating a low binding capacity toward the target protein. Therefore, this compound is considered to have limited potential as a cutinase inhibitor candidate.

Table 4. Two-dimensional (2D) and three-dimensional (3D) visualization of ligand-protein interactions

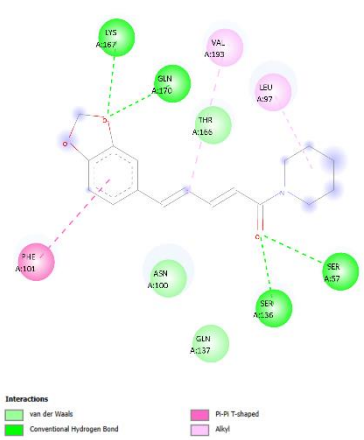
Compounds	2D visualization	3D visualization
Cocrystal ligand		
Mancozeb (Fungicide)		

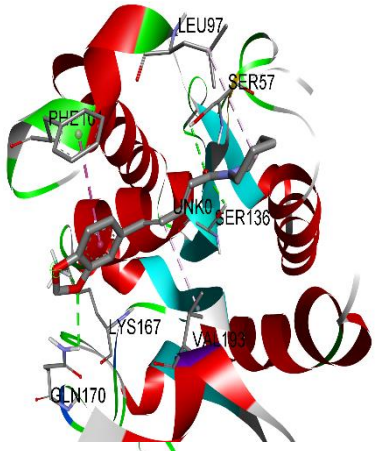
(-)-beta-Sitosterol



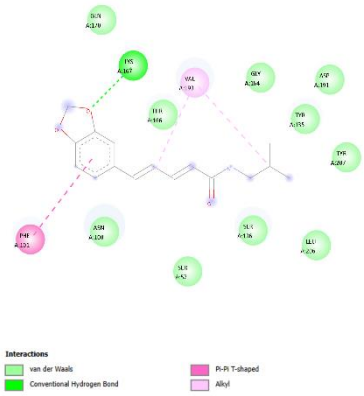


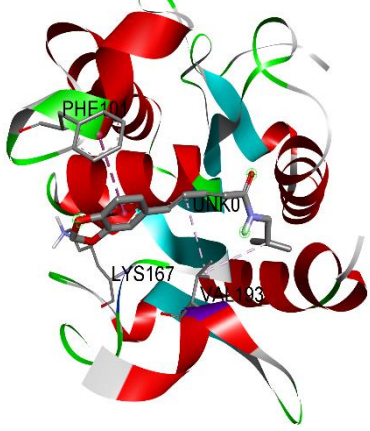
Piperine



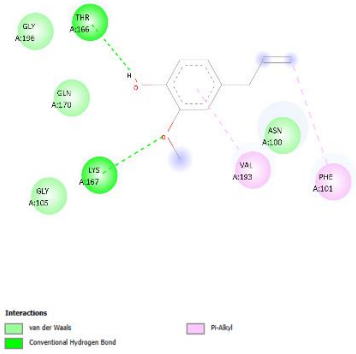


Piperlonguminine





Eugenol





Overall, the results of the Pa–Pi analysis, ADMET analysis, and molecular docking indicate that bioactive compounds from betel leaf have potential as antifungal candidates against the cutinase enzyme of *C. gloeosporioides*, although with varying degrees of suitability. The Pa–Pi analysis showed that all compounds had higher Pa values than Pi values, suggesting potential antifungal activity, with β-sitosterol exhibiting the highest Pa value. However, the ADMET results indicated that some compounds, particularly β-sitosterol, triacontane, and dotriacontanoic acid, had limitations due to their high hydrophobicity and low water solubility, whereas piperine and piperlongumine require further attention because of their higher predicted AMES and carcinogenicity risks. The docking results confirmed that (-)-β-sitosterol had the best affinity for the cutinase enzyme, as indicated by the lowest binding energy and smallest Ki value, followed by piperine and piperlongumine.

In addition, eugenol can also be considered a candidate compound because it exhibits moderate affinity, with a binding energy of -5.1 kcal/mol, and forms hydrogen bonds and hydrophobic interactions. Thus, β-sitosterol can be considered the most promising inhibitor candidate based on docking performance and biological activity prediction, while piperine, piperlongumine, and eugenol have potential as follow-up candidates. However, ADMET aspects, particularly solubility and toxicity, still need to be considered before further in vitro or in vivo testing.

Table 5. Docking parameters and molecular interaction of tested bioactive compounds

Compounds	Binding Energy (Kcal/mol)	Inhibition constant (μm)	Hydrogen Bond	Hydrophobic Interaction
Cocrystal ligand	-4.8	301.60	**Conventional Hydrogen Bond:** Asn100, Lys167 **Carbon Hydrogen Bond**: Thr166	**Van der waals**: Gly105, **Ser136**, Leu97 **Pi-alkyl:** Phe101, Val193
Mancozeb (Fungicide)	-3.2	4497.40	**Conventional Hydrogen Bond:** ASP191, Tyr135	**Van der waals**: Val193, Leu206, Tyr207, Gly164, **Ser36,** Ser66, Phe205, Ala56, **Ser57**
(-)-beta-Sitosterol	-7.1	6.20	-	**Van der waals**: **Ser 57, Ser136,** Ala56, Gly 164, Asn100, Asp191, Thr166, Lys167, Gln170, Gly196 **Alkyl:** Val193, Leu97 **Pi Alkyl:** Phe101, Tyr135
Piperine	-6.7	12.19	**Conventional Hydrogen Bond: Ser57, Ser136,** Lys167, Gln170	**Van der waals**: Asn100, Gln137, Thr166 **Pi-pi T-shaped:** Phe101 **Alkyl:** Val193, Leu97
Piperlonguminine	-6.5	17.08	**Conventional Hydrogen Bond:** Lys167	**Van der waals**: Gln170, Thr166, Gly164, Asp191, Tyr135, Tyr207, **Ser136**, Leu206, **Ser57**, Asn100 **Pi-pi T-shaped:** Phe101 **Alkyl:** Val193
Eugenol	-5.1	181.72	**Conventional Hydrogen:** Lys167	**Van der waals**: Gly196, Gln170, Gly105, Asn100 Pi Alkyl: Val193, Phe101
Carvacrol	-4.5	500.58	**Carbon Hydrogen Bond**: Thr166	**Van der waals**: Asn100, **Ser136**, Gln170, Lys167 **Pi-Pi T-shaped:** Phe101 **Alkyl:** Val193

Chavicol	-4.4	592.68	-	**Van der waals**: Gln170, Lys167, Asn100 **Pi-Sigma**: Thr166 **Pi-Alkyl**: Val193, Phe101
Estragol	-4.3	701.73	**Conventional Hydrogen: Ser136, Ser57**	**Van der waals**: Asn100, Thr166, **Gln137** **Alkyl:** Leu97 **Pi-Alkyl:** Phe101, Val193
Triacontane	-4.1	983.69	-	**Van der waals**: Asp191, Tyr207, Gly164, Phe205, Ala56, **Ser136, Ser57,** Phe101, Thr166, Asn100, Gly196 **Alkyl:** Leu206, Leu97, Val193, Ala192 **Pi-Alkyl:** Tyr135
Dotriacontanoic acid	-0.8	258964.63	-	**Van der waals**: Tyr207, Asp191, **Ser136,** Gly164 **Pi-Alkyl**: Tyr135 **Alkyl**: Val193

CONCLUSION

In conclusion, bioactive compounds from betel leaf show potential as antifungal candidates against the cutinase enzyme of *C. gloeosporioides* based on Pa–Pi, ADMET, and molecular docking analyses. β-Sitosterol demonstrated the greatest potential, as indicated by its highest Pa value, lowest binding energy, and smallest Ki value; therefore, it can be considered the primary inhibitor candidate. Piperine and piperlongumine also exhibited strong affinity, while eugenol can be considered a supporting candidate with moderate affinity. However, some compounds still showed limitations related to solubility, hydrophobicity, and predicted toxicity; therefore, further in vitro and in vivo studies are required to validate their efficacy and safety.

AUTHORS CONTRIBUTIONS

AMP and NHD conceptualized and designed the study. AMP performed compound identification, biological activity prediction, ADMET analysis, and molecular docking simulations. AMP and DER prepared the protein and ligand structures and validated the docking protocol. AMP, ND and HH assisted in data collection, visualization, and interpretation of the molecular interactions. AMP and EA contributed to the analysis of physicochemical and toxicity properties. AMP, NHD, and DER interpreted the results and drafted the original manuscript. All authors reviewed, revised, and commented on the research methodology, data analysis, interpretation of results, and manuscript structure. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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