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ARTICLE

IN SILICO STUDY OF *Bacillus velezensis*-DERIVED VOLATILE COMPOUNDS AS POTENTIAL INHIBITORS OF THE CUTINASE ENZYME FROM *Colletotrichum gloeosporioides* CAUSING ANTHRACNOSE DISEASE

[*Studi In-silico Senyawa Volatil yang Dihasilkan oleh Bacillus velezensis Sebagai Penghambat Potensial Enzim Cutinase dari Colletotrichum gloeosporioides Penyebab Penyakit Antraknosa*]

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ABSRACT

Anthracnose disease caused by *Colletotrichum gloeosporioides* remains a major constraint in horticultural production, particularly in tropical regions. The cutinase enzyme plays an essential role in fungal pathogenicity by facilitating penetration of the host cuticle during the early stages of infection. Volatile organic compounds (VOCs) produced by *Bacillus velezensis* have been reported to exhibit antifungal activity. Inhibiting the cutinase enzyme disrupts the biochemical mechanism used by *C. gloeosporioides* to breach the plant's waxy cuticle. By blocking cutin degradation, this anti-virulence strategy prevents initial infection and tissue invasion. However, information regarding their molecular interactions with cutinase is still limited. This study aimed to evaluate the potential of *B. velezensis*-derived VOCs as inhibitors of the cutinase enzyme from *C. gloeosporioides* using an in-silico approach. Antifungal bioactivity was predicted using PASS Server analysis, while binding affinity and inhibition constant (Ki) values were determined through molecular docking simulations with AutoDock Vina. Molecular interactions between VOC ligands and the target enzyme were analyzed based on their involvement with key active-site residues. The Pa–Pi analysis indicated that most VOCs, particularly terpenoids and medium-chain alcohols, exhibited higher predicted antifungal activity than inactivity. Molecular docking results showed that several VOCs interacted stably with the active site of cutinase, involving key residues Ser57, Ser136, and Gln137. Among the evaluated compounds, β-ionone exhibited the strongest binding affinity, as indicated by the lowest binding energy and inhibition constant. These results suggested that VOCs produced by *B. velezensis*, especially terpenoid compounds, had potential as biocontrol-based antifungal agents through inhibition of cutinase activity and required further validation through in vitro and in vivo studies.

Keywords: Anthracnose, *Bacillus velezensis*, Cutinase, Molecular Docking, Volatile Organic Compounds

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ABSTRAK

Penyakit antraknosa yang disebabkan oleh Colletotrichum gloeosporioides merupakan salah satu kendala utama pada produksi hortikultura, dengan tingkat kehilangan hasil yang tinggi. Enzim kutinase berperan penting sebagai faktor virulensi pada tahap awal infeksi melalui degradasi lapisan kutikula tanaman. Senyawa volatil (volatile organic compounds, VOCs) yang dihasilkan oleh Bacillus velezensis diketahui memiliki aktivitas antifungal, namun mekanisme interaksi molekulernya terhadap enzim kutinase masih belum banyak dikaji. Penelitian ini bertujuan untuk mengevaluasi secara in silico potensi VOC turunan B. velezensis sebagai inhibitor enzim kutinase dari C. gloeosporioides. Prediksi bioaktivitas antifungal dilakukan menggunakan PASS Server, sedangkan afinitas pengikatan dan konstanta inhibisi (Ki) dianalisis melalui simulasi molecular docking menggunakan AutoDock Vina. Interaksi molekuler antara ligan dan protein target dianalisis berdasarkan keterlibatan residu asam amino kunci pada sisi aktif enzim. Hasil analisis Pa–Pi menunjukkan bahwa sebagian besar VOC, terutama dari kelompok terpenoid dan alkohol rantai menengah, memiliki potensi aktivitas antifungal. Hasil docking molekuler menunjukkan bahwa beberapa senyawa mampu berinteraksi secara stabil dengan sisi aktif kutinase melalui residu Ser57, Ser136, dan Gln137. Di antara seluruh senyawa yang diuji, β-ionone menunjukkan afinitas pengikatan tertinggi dengan nilai energi ikatan terendah dan nilai Ki terkecil. Secara keseluruhan, hasil penelitian ini menunjukkan bahwa VOC yang dihasilkan oleh B. velezensis, khususnya kelompok terpenoid, berpotensi dikembangkan sebagai agen antifungal berbasis hayati melalui mekanisme penghambatan enzim kutinase, serta memerlukan validasi lanjutan secara in vitro dan in vivo.

***Kata kunci**: Antraknosa, Bacillus velezensis, Kutinase, Molecular Docking, Senyawa Volatil Organik*

INTRODUCTION

Anthracnose disease caused by the *Colletotrichum gloeosporioides* species complex is one of the major constraints in horticultural crop production, particularly in tropical regions such as Indonesia (Widodo & Hidayat, 2017). This pathogen has been reported to cause yield losses of up to 80% in fruit and vegetable commodities, both during cultivation and postharvest handling, resulting in significant declines in product quality (Salotti *et al*., 2023). Infection by *C. gloeosporioides* occurs through penetration of the host cuticle mediated by extracellular cell wall–degrading enzymes. Among these enzymes, cutinase plays a critical role as a virulence factor by hydrolyzing cutin polymers, thereby facilitating fungal hyphal penetration into epidermal tissues (Arya & Cohen, 2022). Many plant pathogenic fungi, such as *Fusarium*, *Botrytis*, and *Alternaria*, also produce cutinase during host invasion (Arya & Cohen, 2022). In general, cutinase is widely distributed among phytopathogenic fungi and plays an important role in degrading the plant cuticle during the early stages of infection, including in *Colletotrichum* species. Cutinase plays a crucial role throughout multiple stages of infection, including spore adhesion, germination, cuticle penetration, and subsequent colonization of host tissues (Arya & Cohen, 2022). Cutinase is inhibited by several classes of compounds used experimentally and as fungicide. The standard compounds are organophosphate oxons and carbamates (Schmidt *et al*., 2016), covalent serine modifiers (e.g., DFP, boronates) (Walz & Schwack, 2008), buffer components (Tris, MOPS) (Dickman, 1983), natural alkaloids (berberine) (Li *et al*., 2021), benomyl (Köller *et al*., 1982), and various small molecules and natural products. All the standard compounds could be used in docking for control. Volatile organic compounds (VOCs) produced by antagonistic bacteria can suppress fungal pathogens by disrupting cell membrane integrity and inhibiting spore germination and mycelial growth (Choub *et al*., 2022). Accordingly, inhibition of cutinase activity represents a potential molecular strategy for suppressing early-stage infection.

Management of anthracnose disease is still largely dependent on synthetic fungicides. However, continuous and intensive use of chemical fungicides has led to several drawbacks, including the development of pathogen resistance, environmental contamination, and chemical residue accumulation that may adversely affect human health (Ciofini *et al*., 2022). These limitations have encouraged the exploration of environmentally friendly disease management approaches, including biological control agents.

Bacillus velezensis is a rhizosphere-associated bacterium that has been widely reported as a promising biocontrol agent due to its ability to produce various bioactive secondary metabolites, particularly volatile organic compounds (VOCs) (Rabbee *et al*., 2023). These VOCs are low-molecular-weight compounds (<300 Da) that can diffuse through air and soil pores, enabling indirect inhibition of fungal pathogens without direct contact. The VOCs produced by *B. velezensis* comprise diverse chemical groups, such as alcohols, carbonyls, hydrocarbons, aromatic compounds, and sulfur- and nitrogen-containing compounds (Weisskopf *et al*., 2021).Previous studies have demonstrated that VOCs released by *B. velezensis* strain GX0003742 effectively suppressed postharvest anthracnose disease by up to 80% (Xie *et al*., 2026). Volatile compounds produced by *Bacillus subtilis* CF-3 have been reported to suppress the expression of genes associated with virulence factors in *Colletotrichum gloeosporioides*, thereby reducing the pathogen's ability to infect fruit (Liu *et al*., 2025). Therefore, inhibition of cutinase can disrupt fungal penetration and reduce the establishment of infection, making it a promising target for antifungal development.

Although the antifungal activity of *B. velezensis* VOCs has been extensively demonstrated under in vitro conditions, information regarding their molecular interaction with specific fungal target enzymes, particularly cutinase from *C. gloeosporioides*, remains limited. Inhibiting the cutinase enzyme disrupts the biochemical mechanism used by *C. gloeosporioides* to breach the plant's waxy cuticle. By blocking cutin degradation, this anti-virulence strategy prevents initial infection and tissue invasion (Martínez & Maicas, 2021). This approach is highly beneficial as it minimizes fungal resistance and provides a more targeted, environmentally sustainable alternative to conventional fungicides. Since the experimental identification of enzyme inhibitors is generally time-consuming and resource-demanding, in silico approaches, such as molecular docking, provide an efficient alternative for predicting binding affinity, estimating inhibition constants, and identifying potential inhibitors at the active site of target enzymes (Salmaso & Moro, 2018).

Based on these considerations, this study aims to evaluate the potential of volatile compounds derived from *B. velezensis* as inhibitors of the cutinase enzyme from *C. gloeosporioides* using computational approaches. The objectives of this study include predicting the antifungal bioactivity of VOCs using PASS Server analysis, determining binding affinity and inhibition constant (Ki) values through molecular docking, and analyzing molecular interactions between VOCs and key residues at the active site of the cutinase enzyme.

MATERIALS AND METHODS

Selection of Volatile Compounds (VOCs)

Volatile organic compounds (VOCs) used in this study were selected based on previous reports describing bacterial volatile compounds with biological activity against plant pathogens (Xie *et al*., 2026). The selection criteria included the frequency of occurrence in the bacterial volatilome, predicted bioactivity, and diversity of chemical classes, encompassing terpenoids, aldehydes, ketones, esters, alcohols, hydrocarbons, and aromatic derivatives. The chemical structures of the selected compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and used as ligands in the molecular docking analysis.

Preparation of Enzyme Structure and Ligands

The three-dimensional (3D) structure of the target enzyme, *Glomerella cingulata* PETFP-cutinase complex (PDB ID: 3DEA), was retrieved from the Protein Data Bank (PDB; https://www.rcsb.org/structure/3DEA) for subsequent structural and molecular analysis.The selection of the protein structure was based on the availability of a high-resolution crystal structure, enzyme classification, and molecular symmetry (Flores-Castañón *et al*., 2022). The enzyme structure was prepared by removing water molecules, ions, and co-crystallized ligands, followed by the addition of polar hydrogen atoms and partial charges. Key amino acid residues at the active site, namely Ser57, Ser136, and Gln137, were identified based on literature review and binding pocket analysis of the PDB structure. All VOC ligands were subjected to energy minimization to obtain stable conformations, adjusted to physiological pH conditions, and assigned rotatable bonds prior to conversion into a format suitable for molecular docking.

Molecular Docking Simulation

Molecular docking simulations were performed using AutoDock Vina to evaluate the binding affinity between VOC ligands and the target cutinase enzyme. The protein was used in .pdb format and ligands in .sdf format. The grid box was centered on the active site of the enzyme, encompassing key residues Ser57, Ser136, and Gln137. Docking parameters were kept constant for all ligands to ensure comparability of results. The ligand conformation exhibiting the lowest binding energy was selected as the best docking pose for further analysis (Flores-Castañón *et al.* 2022).

Binding Energy and Molecular Interaction Analysis

Binding affinity was assessed based on the binding energy values (kcal/mol) obtained from the docking simulations (Vaidyanathan *et al.,* 2023). The inhibition constant (Ki) was calculated from the binding energy to estimate the inhibitory potential of each compound against the target enzyme (Pandey & Verma, 2022). Ligand–protein interactions were analyzed to identify hydrogen bonds, hydrophobic interactions, π–π interactions, π–alkyl interactions, and van der Waals forces, with particular emphasis on interactions involving the key active-site residues Ser57, Ser136, and Gln137. The relationship between binding energy and the number and type of molecular interactions was analyzed descriptively (Kullmann *et al.*, 2024). The inhibition constant (Ki) was calculated from the binding energy (ΔG) using the following thermodynamic equation (Pandey & Verma, 2022):

$$K_i = e^{\frac{\Delta G}{RT}}$$

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

Data Visualization

Docking results were visualized using two-dimensional (2D) interaction diagrams and three-dimensional (3D) structural representations to illustrate ligand orientation within the enzyme active site. Binding energy values, inhibition constants (Ki), and interacting amino acid residues were summarized in tables and figures to facilitate interpretation of the relationship between VOC chemical classes and binding affinity.

RESULTS AND DISCUSSION

Bioinformatic Prediction of Antifungal Activity Based on Pa-Pi Analysis and Chemical Class Distribution of Volatile Compounds Produced by *Bacillus velezensis*

The volatile compounds analyzed in Table 1 comprise compounds that have been reported in previous studies as major components of *B. velezensis* VOCs with antifungal activity (Xie *et al.*, 2026). The chemical class distribution of these volatile compounds is further visualized in Figure 1 to illustrate the dominance patterns of compound classes commonly reported in the literature. The distribution of the identified VOCs classes indicates a predominance of terpenoids, followed by aldehydes/ketones/esters and alcohols and amines, which are consistently reported as the principal components of VOCs produced by *B. velezensis*. The dominance of terpenoids in this VOC profile is consistent with previous findings showing that terpenoid compounds exhibit strong antifungal activity by targeting fungal cell membranes. In this mechanism, the presence of phenolic –OH groups and hydrophobic properties plays a critical role in penetrating and disrupting the phospholipid bilayer, ultimately compromising fungal cell membrane integrity (Konuk & Ergüden, 2020).

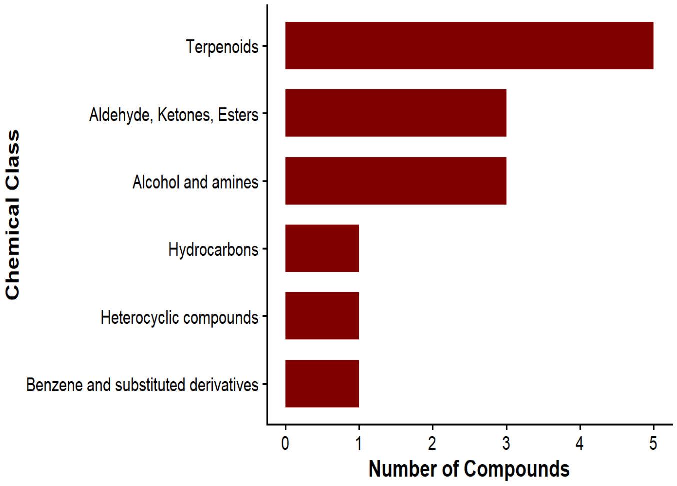


Figure 1. Chemical Class Distribution of Volatile Organic Compounds Produced by *B. velezensis (Distribusi Kelas Kimia Senyawa Organik Volatil yang Diproduksi oleh B. velezensis*).

Bioinformatic predictions based on the probability of activity (Pa) and probability of inactivity (Pi) values indicate that all identified volatile compounds produced by *B. velezensis* exhibit higher Pa than Pi values, suggesting their potential antifungal activity. The terpenoid group, including citronellol, linalool, geraniol, β-ionone, and trans-β-ocimene, demonstrated relatively high Pa values (0.542–0.596) accompanied by very low Pi values (0.019–0.024), indicating a strong and consistent prediction of antifungal potential. The predicted biological activities obtained from PASS were interpreted based on the probability of activity (Pa). A Pa value > 0.7 indicates strong probability, 0.5–0.7 indicates moderate probability, and Pa < 0.5 indicates weak probability of biological activity, suggesting a lower likelihood of experimental confirmation (Bashera *et al.*, 2025). These findings are consistent with previous reports showing that the terpenoid compound citronellal can inhibit the growth of *Penicillium digitatum* by reducing cell membrane integrity through the inhibition of ergosterol biosynthesis, thereby disrupting fungal membrane structure (OuYang *et al.*, 2021).

Table 1. Volatile Organic Compounds Produced by *B. velezensis* with Reported Antifungal Activity (*Senyawa Organik Volatil yang Diproduksi oleh B. velezensis dengan Aktivitas Antijamur yang Dilaporkan*).

Compounds (*Senyawa*)	**Class** (*Kelompok*)	**CAS**	**Pa**	**Pi**	**Activity** (*Aktivitas*)
Nystatin (Positive Control)	Polyketide (*poliketida*)	1400-61-9	0.986	0.000	Antifungal (*antijamur*)
heptanoic acid, ethyl ester	Aldehyde, Ketones, Esters (*Aldehida, Keton, Ester*)	106-30-9	0.396	0.050	Antifungal (*antijamur*)
Benzeneacetaldehyde	Aldehyde, Ketones, Esters (*Aldehida, Keton, Ester*)	122-78-1	0.238	0.113	Antifungal (*antijamur*)
Nonanal	Aldehyde, Ketones, Esters (*Aldehida, Keton, Ester*)	124-19-6	0.314	0.075	Antifungal (*antijamur*)
Citronellol	Terpenoids (*terpenoid*)	106-22-9	0.585	0.020	Antifungal (*antijamur*)
Linalool	Terpenoids (*terpenoid*)	78-70-6	0.596	0.019	Antifungal (*antijamur*)
Geraniol	Terpenoids (*terpenoid*)	106-24-1	0.563	0.022	Antifungal (*antijamur*)
β-ionone	Terpenoids (*terpenoid*)	79-77-6	0.542	0.024	Antifungal (*antijamur*)
trans-b-ocimene	Terpenoids (*terpenoid*)	3779-61-1	0.573	0.021	Antifungal (*antijamur*)

Compounds (*Senyawa*)	**Class** (*Kelompok*)	**CAS**	**Pa**	**Pi**	**Activity** (*Aktivitas*)
Pyrazine, trimethyl-	Heterocyclic compounds (*Senyawa heterosiklik*)	14667-55-1	0.166	0.165	Antifungal (*antijamur*)
Phenylethyl alcohol	Alcohol and amines (*alkohol dan amina*)	60-12-8	0.275	0.093	Antifungal (*antijamur*)
2-nonanol	Alcohol and amines (*alkohol dan amina*)	628-99-9	0.523	0.027	Antifungal (*antijamur*)
2-undecanol	Alcohol and amines (*alkohol dan amina*)	1653-30-1	0.523	0.027	Antifungal (*antijamur*)
Tridecane	Hydrocarbons (*hidrokarbon*)	629-50-5	0.377	0.055	Antifungal (*antijamur*)
2-methoxy-phenol	Benzene and substituted derivatives (*Benzena dan turunan pengganti*)	90-05-1	0.353	0.062	Antifungal (*antijamur*)

Aldehyde, ketone, and ester compounds, including heptanoic acid ethyl ester, benzeneacetaldehyde, and nonanal, exhibited moderate antifungal potential, with Pa values ranging from 0.238 to 0.396 and relatively low Pi values. Non-terpenoid volatile compounds produced by microorganisms—such as ketones, alcohols, and esters—have been reported to inhibit the growth of soil-borne pathogenic fungi by reducing the activity of cell wall–degrading enzymes and disrupting the regulation of key pathogenicity-related genes, thereby contributing to synergistic VOC-mediated inhibition mechanisms (Kong *et al*., 2022). In addition, medium-chain alcohol compounds, particularly 2-nonanol and 2-undecanol, showed high Pa values (>0.52), further supporting their potential roles as effective antifungal agents through the inhibition of fungal hyphal growth.

In contrast, heterocyclic compounds such as trimethylpyrazine exhibited nearly balanced Pa and Pi values, indicating limited antifungal potential when acting individually. However, these compounds are more likely to function as signaling molecules or modulators of microbial interactions rather than as primary antifungal agents. Overall, the Pa–Pi analysis suggests that the predicted antifungal activity arises from the collective action of multiple volatile compounds, with terpenoids and medium-chain alcohols serving as the primary contributors, while aldehydes, esters, hydrocarbons, and phenolic derivatives play supportive roles by synergistically enhancing the overall antifungal effect. These findings indicate that the antifungal potential of the volatile compound profile is multicomponent and multitargeted, thereby strengthening its prospects as an environmentally friendly source of natural antifungal agents.

Molecular Docking Analysis of Volatile Compounds Reveals Their Bindung Interactions with Cutinase Enzymes

Pathogenic fungi such as *Colletotrichum gloeosporioides* are major causal agents of anthracnose disease in a wide range of horticultural commodities, particularly fruits (Guo *et al*., 2022; Sharma & Kulshrestha, 2015). The plant cuticle serves as the first physical barrier against pathogen invasion; therefore, successful infection requires pathogens to penetrate this protective layer to colonize host tissues. This process is primarily mediated by cutinase, an extracellular enzyme that hydrolyzes ester bonds within cutin polymers. In *Colletotrichum* species, the production and secretion of cutinase are key determinants of host cuticle degradation and are essential for successful penetration and progression into the necrotrophic phase of infection (Villafana & Rampersad, 2020). Specifically, *Colletotrichum truncatum* infects host plants through direct cuticle penetration mediated by the cutinase gene *CtCut1*, which has been shown to play an essential role in pathogenicity and disease development (Auyong *et al*., 2015). Thus, cutinase not only contributes to the initial penetration process but also plays a crucial role in determining pathogen virulence and the development of anthracnose disease, making it a strategic target for disease control through the inhibition of virulence-related enzymes. Accordingly, this study was conducted in silico to evaluate the potential of bioactive compounds as cutinase inhibitors by analyzing their molecular interactions and binding affinities at the enzyme’s active site.

In line with the objective of this in silico study to evaluate the inhibitory potential of bioactive compounds against cutinase, molecular docking analysis revealed that the amino acid residues Ser57 (S57), Ser136 (S136), and Gln137 (Q137) are key residues involved in the formation and stabilization of ligand–cutinase complexes in *C. gloeosporioides* (Nyon *et al.*, 2009). These residues were consistently engaged in interactions with various volatile compounds, primarily through the formation of conventional hydrogen bonds that contribute substantially to complex stability. The serine residues S57 and S136, which possess reactive hydroxyl groups, facilitate strong polar interactions with ligand functional groups, thereby helping to maintain proper ligand orientation within the binding pocket. In contrast, the glutamine residue Q137 contributes mainly through van der Waals interactions and weak polar contacts, reinforcing the spatial accommodation and overall stability of the ligand within the active site of the target enzyme. Compounds exhibiting low binding energy and small inhibition constant (Ki) values generally showed direct interactions with one or more of these key residues, confirming that ligand binding at these positions has the potential to interfere with the catalytic activity of the target enzyme. Variations in Ki values among a series of compounds can be converted into changes in binding free energy (ΔΔG), thereby enabling a direct correlation between ligand structural features and specific interactions within the enzyme binding site (Copeland, 2005).

The docking protocol was validated by redocking the cocrystal ligand into the active site of the target protein. The resulting root mean square deviation (RMSD) value between the docked pose and the native ligand conformation was 1.475 Å, indicating that the docking method is reliable since the RMSD value is below 2.0 Å. Molecular docking results demonstrated that the tested volatile compounds exhibited varying binding affinities toward the cutinase enzyme, as reflected by differences in binding energy and inhibition constant (Ki) values (Table 2). Among the analyzed compounds, β-ionone showed the highest binding affinity, characterized by the lowest binding energy (−5.0 kcal/mol) and a relatively low Ki value (215.15 µM), indicating a more stable interaction than that of the native ligand. These results suggest that β-ionone has the potential to act as a competitive inhibitor of cutinase. Notably, a thiazolylhydrazone derivative of β-ionone has been successfully synthesized and reported to exhibit strong antioxidant and antifungal activities against plant pathogens in vitro (Huang *et al.*, 2023). Interaction analysis further revealed that β-ionone binds directly to the key catalytic residue Ser136 (S136) within the cutinase active site through hydrogen bond formation, which is critical for maintaining ligand orientation within the enzyme binding pocket. Given the essential role of Ser136 in the hydrolytic mechanism of cutinase, ligand binding at this residue may interfere with enzymatic activity during host cuticle degradation in the infection process. In addition, hydrophobic interactions and van der Waals contacts with surrounding nonpolar residues, including Ser57, contribute to stabilizing the ligand–enzyme complex.

Other terpenoid compounds, including geraniol, citronellol, and trans-β-ocimene, also exhibited relatively stable binding affinities toward the cutinase enzyme, as indicated by moderately low binding energy values (−4.1 to −4.4 kcal/mol). These results suggest that these compounds can interact consistently with the enzyme's active site. In contrast, compounds with long-chain hydrocarbon structures, such as tridecane, displayed weaker binding affinities, as reflected by less negative binding energy values and higher Ki values, indicating a lower potential for effective cutinase inhibition. Analysis of the interaction patterns revealed that the stability of the ligand–enzyme complex is primarily governed by a combination of hydrogen bonding and hydrophobic interactions, including van der Waals, alkyl, and π-alkyl contacts.

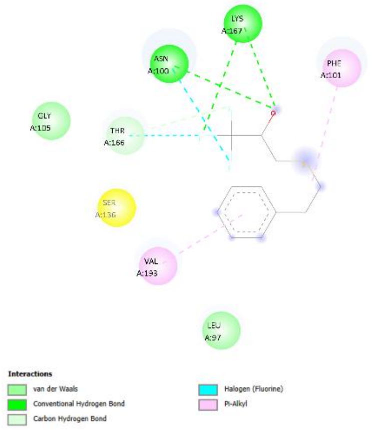
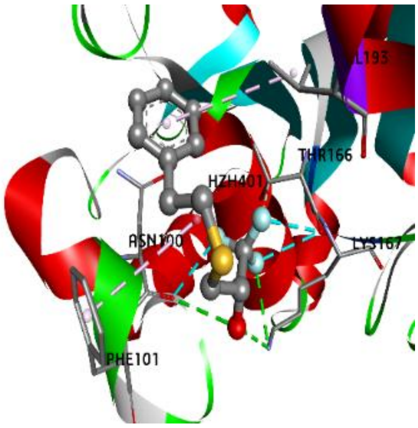
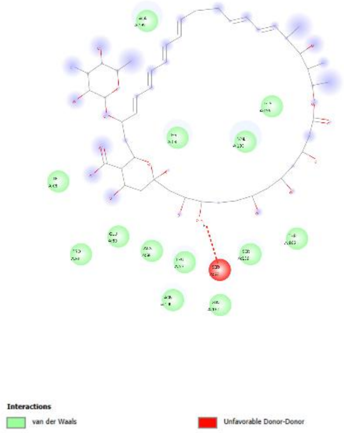
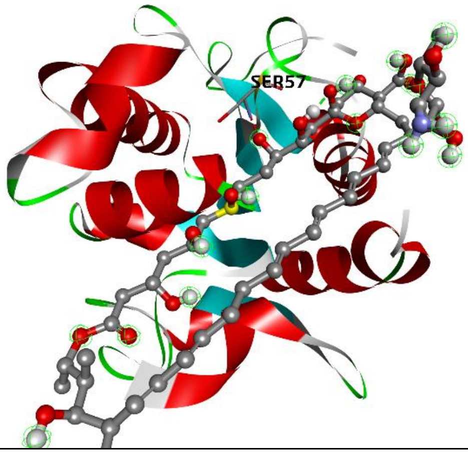
Table 2. Docking parameters and molecular interaction of tested compounds (*Parameter docking dan interaksi molekuler senyawa yang diuji*).

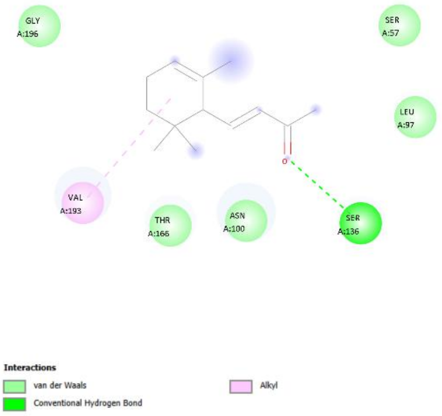
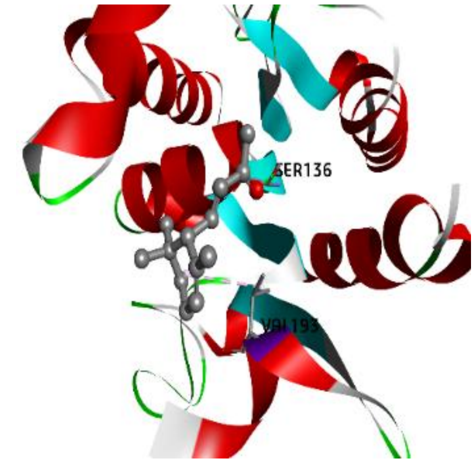
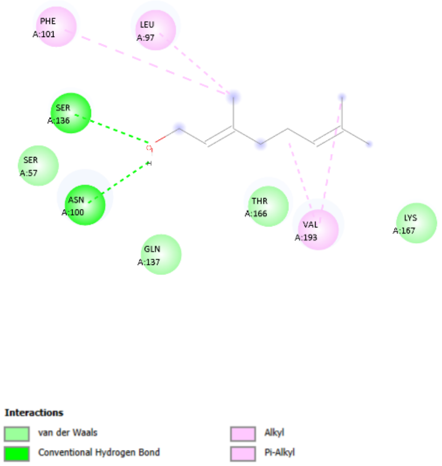
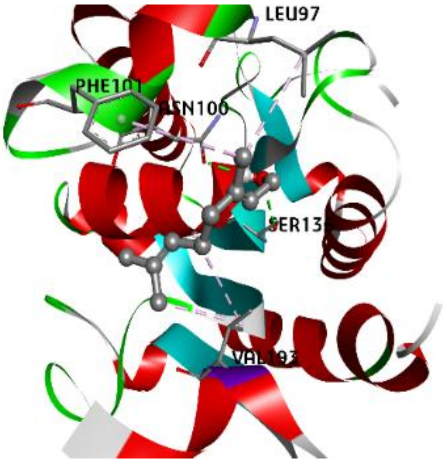
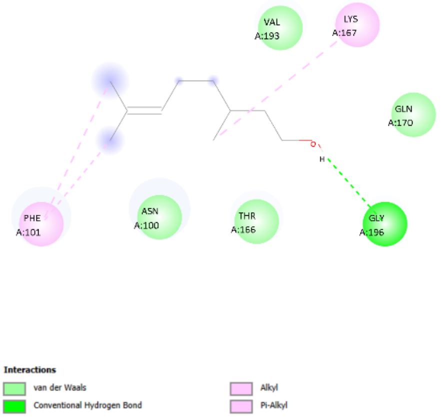
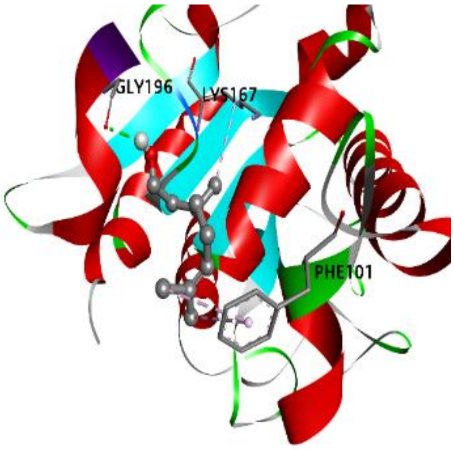
Compounds (*Senyawa*)	**Binding Energy** (*Enegi Ikatan*) **(Kcal/mol)** (Kkal/mol)	**Inhibition constant** (*Kosntanta Inhibisi*) **(µm)**	**Hydrogen Bond** (*Ikatan Hidrogen*)	**Hydrophobic Interaction** (*Interaksi Hidrofobik*)
Cocrystal ligand (*ligan kokristal*)	-4.8	301.60	**Conventional Hydrogen Bond:** Asn100, Lys167 **Carbon Hydrogen Bond**: Thr166	**Van der waals**: Gly105, **Ser136**, Leu97 **Pi-alkyl:** Phe101, Val193
Nystatin (Positive control) (*Kontrol positif*)	-4,5	502.50	**Unfavorable donor-donor: Ser57**	**Van der waals:** Ile65, Pro60, Glu59, Ala56, Leu97, Asn100, **Gln137**, **Ser136,** Thr166, Thr50, Val193, Gly196
β-ionone	-5.0	215.15	**Conventional Hydrogen Bond: Ser136**	**Van der waals:** Gly196, Thr166, Asn100, Leu97, **Ser57** **Alkyl:** Val193
Geraniol	-4.4	592.68	**Conventional Hydrogen Bond: Ser136**, Asn100	**Van der waals: Ser57, Gln137**, Thr166, Lys167 **Alkyl:** Phe101, Leu97, Val193
Citronellol	-4.2	830.83	**Conventional Hydrogen Bond**: Gly196	**Van der waals:** Asn100, Thr166, Gln170, Val193 **Alkyl:** Phe101, Lys167
Phenylethyl alcohol	-4.2	830.83	**Conventional Hydrogen Bond: Ser136**, **Ser57**	**Van der waals:** Asn100, **Gln137**, Leu97, Val193, Thr166 **Pi-pi T-shaped:** Phe101
trans-β-ocimene	-4.1	983.68	-	**Van der waals:** Lys167, Thr166, Asn100 **Alkyl:** Val193, Phe101, Leu97
Benzeneacetaldehyde	-4.1	983.68	**Conventional Hydrogen Bond: Ser57, Ser136**	**Van der waals: Gln137**, Leu97, Val193, Asn100, Thr166 **Pi-pi T-shaped:** Phe101
2-methoxy-phenol	-4.0	1164.66	**Conventional Hydrogen Bond:** Asn100, Lys167, Gln170	**Van der waals:** Gly196, Thr166, Gly105, Phe101 **Pi-alkyl:** Val193
Linalool	-4.0	1164.66	-	**Van der waals:** Asn100, Thr166, Gln170, Gly196, Lys 167 **P1-sigma:** Phe101, **Alkyl:** Val193
Pyrazine, trimethyl-	-3.8	1632.64	**Conventional Hydrogen Bond: Ser136** **Pi-donor Hydrogen Bond:** Tyr135	**Van der waals:** Asp191, Gly164 **Alkyl:** Val193, Ala56, Leu206 **Pi Alkyl:** Tyr207
heptanoic acid, ethyl ester	-3.8	1632.64	**Conventional Hydrogen Bond: Ser136,** Tyr135	**Van der waals:** Asp191, Gly194, Tyr207, Phe205, Ser66, **Ser57,** Glu59 **Alkyl:** Val193, Ala56
2-nonanol	-3.6	2288.66	**Conventional Hydrogen bond:** Val193, Thr166	**Van der waals:** Asn100, Lys167, Gln170, Gly196, **Alkyl:** Phe101
Nonanal	-3.6	2288.66	**Conventional Hydrogen bond: Ser57, Ser136**	**Van der waals: Gln137**, Thr58, Glu59, Ser66, Ile65, **Pi-Alkyl:** Phe205, Ala56, Tyr135
2-undecanol	-3.6	2288.66	-	**Van der waals:** Asn100, Gln170, Gly196, Thr166, Lys167 **Alkyl:** Val193 **Pi-alkyl:** Phe101

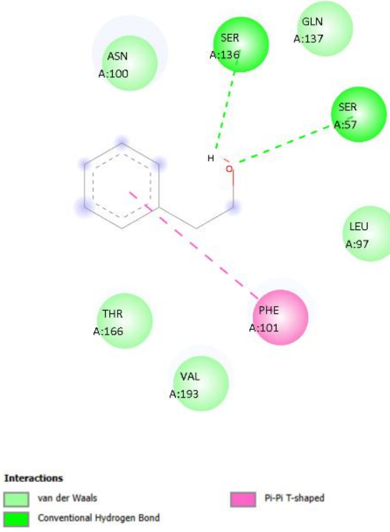
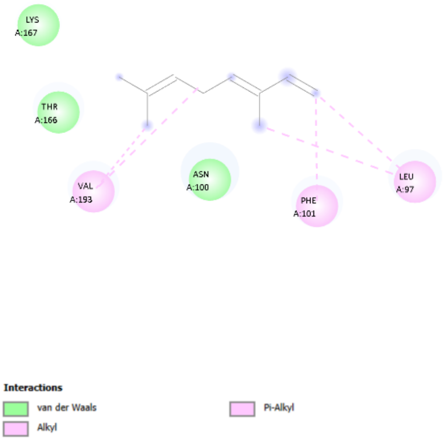
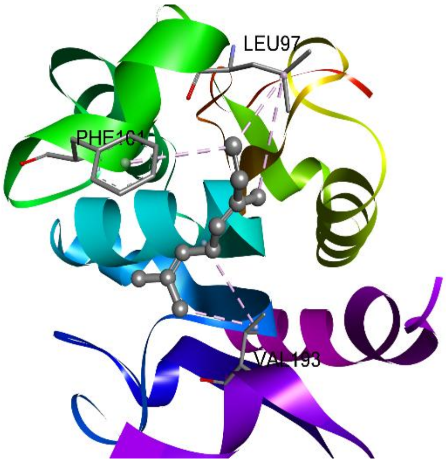
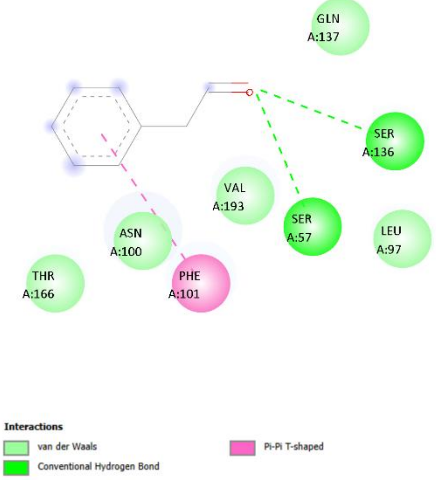
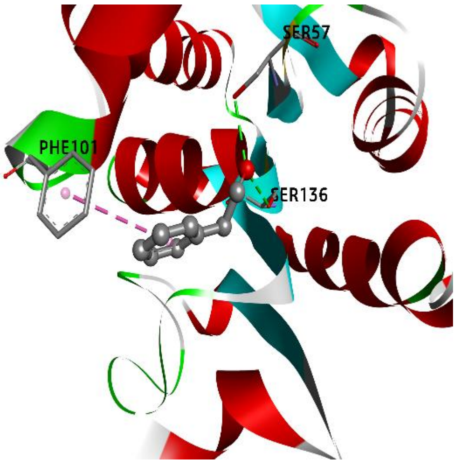
Compounds (*Senyawa)*	**Binding Energy** (*Enegi Ikatan*) **(Kcal/mol)** (Kkal/mol)	**Inhibition constant** (*Kosntanta Inhibisi*) **(µm)**	**Hydrogen Bond** (*Ikatan Hidrogen*)	**Hydrophobic Interaction** (*Interaksi Hidrofobik*)
Tridecane	-3.5	2709.73	-	**Van der waals:** Gly196, Lys167, Gln170, Thr166, Asn100, **Ser57**, Leu97 **Alkyl:** Val193, **Pi-alkyl:** Phe101

Two-dimensional (2D) and three-dimensional (3D) docking visualizations further support these quantitative findings (Table 3), showing that ligands with the lowest binding energy values are stably accommodated within the binding pocket of the target protein, with orientations comparable to those of the native ligand. Among the tested compounds, β-ionone and geraniol exhibited multipoint interactions with key residues surrounding the active site, facilitating the formation of more stable ligand–protein complexes. Overall, the integration of binding energy values, Ki data, amino acid residue interaction profiles, and docking visualizations provides strong structural evidence that volatile compounds—particularly those belonging to the terpenoid group—have the potential to inhibit target protein function and contribute to the bioinformatically predicted antifungal activity against *C. gloeosporioides*.

Table 3. Two-dimensional (2D) and three-dimensional (3D) visualization of ligand-protein interactions (*Visualisasi interaksi ligan-protein dua dimensi (2D) dan tiga dimensi (3D))*

Compounds (*Senyawa*)	**2D visualization** (*Visualisasi 2D*)	**3D visualization** (*Visualisasi 3D*)
Cocrystal ligand (*ligan kokristal)*)		
Nystatin (Positive control) (*kontrol positif*)		

Compounds (*Senyawa*)	**2D visualization** (*Visualisasi 2D*)	**3D visualization** (*Visualisasi 3D*)
β -ionone		
Geraniol		
Citronellol		
Phenylethyl alcohol		

Compounds (*Senyawa*)	**2D visualization** (*Visualisasi 2D*)	**3D visualization** (*Visualisasi 3D*)
		
trans-β-ocimene		
Benzeneacetaldehyde		

Relationship between Binding Energy and Inhibition Constant (Ki)

The graph demonstrates a clear linear relationship between binding energy and inhibition constant (Ki) values (Figure 2), in which decreasing binding energy (i.e., more negative values) is associated with decreasing Ki values. It is stated that highly potent inhibitors are frequently in the low nanomolar (nM) range (e.g., = 0.011 nM – 5.8 nM), moderate inhibitors are often in the micromolar (µM) range (e.g., 1–100 µM), and weak inhibitors could be above 100 µM or in the mM range (Cournia *et al*., 2017; Goettig *et al*., 2024; Zheng & Polli, 2010). Compounds exhibiting the lowest binding energies, such as β-ionone (−5.0 kcal/mol), displayed the smallest Ki values (approximately 215 µM), indicating the weak to moderate binding affinity toward the cutinase enzyme. Nevertheless, β-ionone has been reported to exhibit in vitro antifungal activity against *Aspergillus fumigatus* and to inhibit fungal growth and toxin production (Yin *et al*., 2022), suggesting that this compound may contribute to antifungal effects through additional biological mechanisms beyond strong target binding. In contrast, compounds with higher (less negative) binding energy values showed substantially increased Ki values, exceeding 2000 µM, which reflects weaker binding affinities.

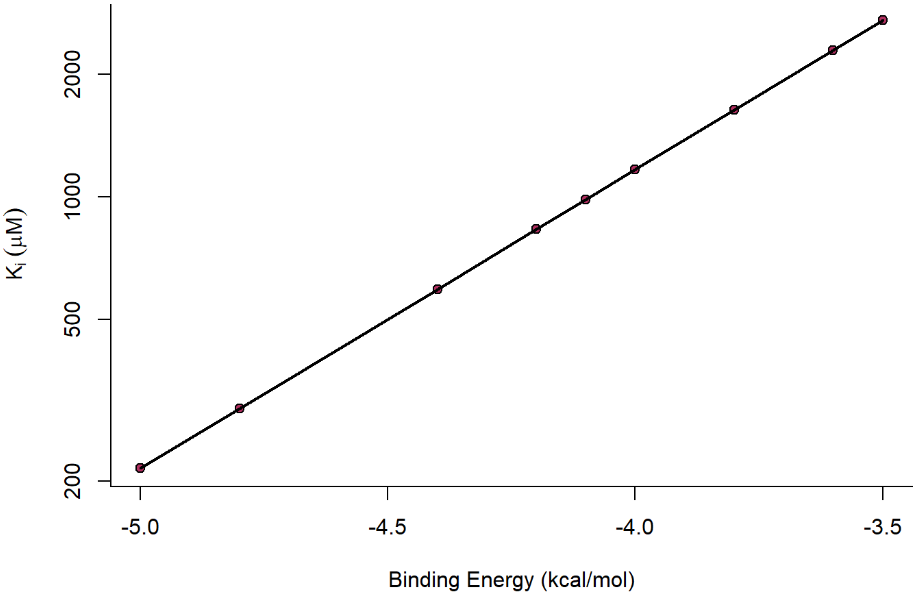


Figure 2. Relationship between binding energy and Ki (*Hubungan antara energi pengikat dan Ki*).

This relationship confirms that the molecular docking results are thermodynamically consistent, with lower binding free energy directly corresponding to greater inhibitory capacity. The observed linear pattern further indicates that differences in binding affinity among compounds are primarily governed by the strength and number of interactions between ligands and key active-site residues of the cutinase enzyme, particularly Ser57, Gln137, and Ser136. Consequently, this graph reinforces the reliability of the docking analysis and supports the interpretation that compounds forming specific interactions with these critical residues possess enhanced inhibitory potential. Binding affinity between proteins and ligands is widely used as a key indicator for evaluating potential inhibitors, as stronger ligand–protein interactions generally correspond to greater inhibitory effects and improved binding stability (Yang *et al*., 2024).

CONCLUSION

This silico study demonstrates that volatile organic compounds (VOCs) produced by *B velezensis* have potential as inhibitors of the cutinase enzyme from *C. gloeosporioides*, the causal agent of anthracnose disease. Pa–Pi analysis indicated that most VOCs, particularly terpenoids and medium-chain alcohols, exhibited predicted antifungal activity. Molecular docking results revealed that several VOCs interacted stably with the active site of the cutinase enzyme, involving key amino acid residues Ser57, Ser136, and Gln137. Among the evaluated compounds, β-ionone showed the highest binding affinity, as reflected by the lowest binding energy and inhibition constant (Ki) values. The association between binding energy and Ki values supports the consistency of the molecular docking analysis. Overall, these findings suggest that VOCs derived from *B. velezensis*, especially terpenoid compounds, have potential to be developed as biocontrol-based antifungal agents. However, further validation through in vitro and in vivo studies is required to confirm their efficacy and practical applicability.

AUTHOR CONTRIBUTIONS

AMP: Create research concepts, collecting research data, drafting the article, final revision of the manuscript; AK: Draft articles, revise manuscripts; RRD: Draft articles, revise manuscripts.

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