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In Silico Study: Evaluation of the Potential of Turmeric Extract Curcumin as an Inhibitor Agent of *Ganoderma boninense* to Mitigate the Decline in CPO Production in Indonesia

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ABSTRACT
Oil palm is a strategic commodity for Indonesia, contributing up to 56% of global *Crude Palm Oil* (CPO) exports since 2020. However, *Basal Stem Rot* (BSR) disease caused by *Ganoderma boninense* remains a major threat, with potential economic losses estimated at USD 50–350 million annually. The synthetic fungicide hexaconazole has long been applied for control, but it poses risks of soil toxicity and disruption of the nitrogen cycle. This study aimed to evaluate the potential of curcumin as a natural fungicidal agent through an in silico approach. Three target enzymes, namely laccase, manganese peroxidase, and lignin peroxidase, were analyzed using *molecular docking*. Protein structures were retrieved from RCSB PDB, while the curcumin ligand was obtained from PubChem. Molecular preparation was performed with BIOVIA Discovery Studio, docking with PyRx, and visualization with PyMOL. Results showed binding affinities of -6.53, -9.17, and -9.00 kcal/mol, respectively, with valid RMSD values (lower <2; upper <5), indicating stable interactions at the protein active sites. These findings suggest that curcumin has strong potential as an eco-friendly inhibitor of *G. boninense* and may serve as a natural alternative to hexaconazole for BSR management in oil palm.

ABSTRAK
Kelapa sawit merupakan komoditas strategis Indonesia yang sejak 2020 mendominasi 56% ekspor global Crude Palm Oil *(CPO). Namun, penyakit* Basal Stem Rot *(BSR) akibat infeksi* Ganoderma boninense *masih menjadi ancaman utama dengan potensi kerugian 50–350 juta USD per tahun. Fungisida sintetis hexaconazole telah lama digunakan, tetapi berisiko menimbulkan toksisitas tanah dan mengganggu siklus nitrogen. Penelitian ini bertujuan mengevaluasi potensi kurkumin sebagai agen fungisida alami melalui pendekatan* in silico. *Tiga enzim target, yaitu lakase, mangan peroksidase, dan lignin peroksidase, dianalisis menggunakan metode* molecular docking *dengan struktur protein dari RCSB PDB dan ligan kurkumin dari PubChem. Preparasi molekul dilakukan dengan BIOVIA* Discovery Studio*, penambatan menggunakan PyRx, sedangkan hasil visualisasi dianalisis melalui PyMOL. Hasil menunjukkan* binding affinity *kurkumin masing-masing sebesar -6,53; -9,17; dan -9,00 kkal/mol dengan nilai RMSD valid* (lower <2; upper <5)*, yang mengindikasikan ikatan stabil pada sisi aktif protein. Dengan demikian, kurkumin terbukti berpotensi sebagai inhibitor G. boninense yang ramah lingkungan dan dapat menjadi alternatif pengganti hexaconazole untuk pengendalian BSR pada kelapa sawit.*

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INTRODUCTION

Indonesia has experienced a drastic increase in oil palm plantation area from year to year. This condition is supported by oil palm being an important tropical export crop with a high oil yield, approximately 4 tons per hectare per year (Zhao et al., 2023). In 2022, Indonesia cultivated 14.5 million hectares of oil palm plantations and produced 46 million metric tons of crude palm oil (CPO) (Arif, 2024). The palm oil industry plays a vital role in the national economy, contributing 13.5% of non-oil and gas exports and 3.5% of the country's gross domestic product (GDP) (Zuhdi et al., 2021). Palm oil is widely utilized as a primary raw material in the food, biodiesel, and oleochemical industries. However, oil palm is susceptible to various diseases, particularly *Basal Stem Rot* (BSR) caused by *Ganoderma boninense* (Santosa, 2020). Infections of *Ganoderma* in oil palm plantations have become a critical issue threatening the sustainability of the national palm oil industry (Wirianata et al., 2022). BSR caused by *G. boninense* has spread widely, resulting in substantial economic losses. The disease can reduce plantation yields by 50–80% and shorten the economic lifespan of oil palm trees by up to 50% (Yusuf, 2024).

Ganoderma boninense is the primary pathogen causing basal stem rot (BSR) in oil palm, characterized by its strong ability to degrade lignin (Ramzi et al., 2019). This activity is mainly supported by three key ligninolytic enzymes belonging to the class II peroxidases and multicopper oxidases, namely manganese peroxidase (MnP), lignin

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peroxidase (LiP), and laccase (Lac) (Siddiqui et al., 2019). Structurally, MnP in *G. boninense* contains conserved motifs of aspartate and histidine, which are crucial for binding Mn^{2+} ions (Ho et al., 2020). This enables the oxidation of Mn^{2+} to Mn^{3+}, which subsequently acts as a mediator to attack lignin's aromatic bonds, accelerating the fragmentation of the lignocellulosic polymer (Danilovich et al., 2021). Expression of MnP is reported to increase under conditions rich in complex carbon sources, highlighting its importance when the fungus encounters rigid lignin matrices in plant tissues. Meanwhile, LiP in *G. boninense* is characterized by the conserved tryptophan-171 motif and a heme domain, enabling it to oxidize non-phenolic substrates with high redox potential (Singh et al., 2020). This provides the fungus with the advantage of breaking aryl–ether linkages in lignin, which are typically resistant to conventional peroxidases. Transcriptomic studies further revealed that LiP expression is upregulated during direct interaction with oil palm root tissues, suggesting its role in early penetration and colonization (Ho et al., 2020). Laccase (Lac), on the other hand, is characterized by four copper-binding centers (Cu T1, Cu T2, and a pair of Cu T3) that facilitate electron transfer. These sites allow Lac to oxidize both phenolic and non-phenolic substrates, with the aid of small redox mediators (Ramzi et al., 2019). In *G. boninense*, Lac isoforms are expressed during the colonization phase, playing a dual role in detoxifying plant defense-related phenolic compounds and synergistically supporting lignin degradation together with MnP and LiP. Comparative genomic analyses have also revealed a significant expansion of carbohydrate-active enzyme (CAZyme) families in *G. boninense*, particularly AA1 (laccase) and AA2 (peroxidases, including MnP and LiP). This highlights that these three ligninolytic enzymes do not act independently, but instead function synergistically to enhance the fungus's ability to degrade complex lignocellulosic substrates. Collectively, the activities of MnP, LiP, and Lac not only support lignin degradation but also play a strategic role in the pathogenicity of *G. boninense* toward oil palm (Bharudin et al., 2022; Utomo et al., 2024).

Methods to control *G. boninense*, such as the application of synthetic pesticides, including hexaconazole, have been the options. Although considered effective against various plant diseases, this chemical control raises concerns. (Jellali et al., 2021). Additional challenges for preventing BSR in oil palm have been reported that hexaconazole pressure injection for BSR management is deemed impractical due to high equipment costs and the need for skilled labour. Beyond these cost constraints, some reports indicate that hexaconazole-based control of BSR in oil palm has been largely neglected or ineffective (Jazuli *et al.*, 2022). This situation underscores the need for alternative control measures that are safer and more environmentally friendly for managing *G. boninense* in oil palm, while maintaining oil production sustainability and ecosystem health (Arif, 2024).

Turmeric (*Curcuma longa*) produces curcumin, a compound long recognised in both traditional and modern medicine for its broad spectrum of biological activities, including antioxidant, anti-inflammatory, anticancer, and antifungal properties (Hajifathali *et al.*, 2023). *Curcuma longa* and its polyphenolic compound curcumin have demonstrated antimicrobial activity with relatively few side effects. Studies have further indicated that curcumin exhibits stronger antifungal potential compared to synthetic pesticides such as hexaconazole (Wang, Y., et al., 2018). The main advantages of curcumin over chemical fungicides lie in its natural origin, biodegradability, and low toxicity to mammals and the environment, making it an ideal candidate for the development of sustainable biocontrol agents (Adnan & Soeparno, 2021). In controlling *Ganoderma boninense*, multiple scientific reports have highlighted the promising potential of curcumin as an inhibitor. One study shows Curcumin inhibits fungal growth by disrupting cell membrane integrity through the suppression of ergosterol synthesis, impairing mitochondrial respiration by reducing NADH oxidase and succinate dehydrogenase activity, and interfering with essential energy and protein metabolism by downregulating key enzymes such as GAPDH and tRNA-synthetase. These combined effects destabilize the fungal cell, reduce ATP production, and ultimately lead to cell death (Chen *et al.*, 2018). Its proven antifungal activity against various fungal species provides a strong scientific basis for further exploration of its efficacy in suppressing *G. boninense* infections (Chintakovid, 2022). Even more promising, at specific concentrations, curcumin has been reported to be as effective as conventional synthetic fungicides such as hexaconazole (Adnan & Soeparno, 2021). The in silico method is a modern research approach based on computational data analysis using computer systems, complementing conventional in vivo (within living organisms) and in vitro (in glass/laboratory) methods (Khaerunnisa, 2023). Although relatively new, this approach is increasingly applied in fields such as medicine, biotechnology, and health sciences due to its ability to utilise bioinformatics and biological databases to solve problems virtually (Rahmani et al., 2022). In pharmaceutical and chemical research, the use of test animals has been increasingly restricted due to time, cost, and ethical considerations. Consequently, in silico methods are gaining attention as an alternative, since they can be conducted more rapidly and at significantly lower costs conventional experiments (Makatita, 2020).

The advantages of in silico methods make them highly attractive for scientific research and drug discovery. First, in silico significantly reduces research costs and saves time, as computer simulations can provide predictive results quickly without the need for expensive reagents or biological samples (Makatita, 2020; Fernández-de Córdoba et al., 2025). Second, this approach does not require the physical isolation of compounds or organisms at the early stage of research, allowing the potential benefits of a compound to be evaluated before synthesis or testing in vitro or in vivo assays (Fernández-de Córdoba et al., 2025). This enables high-throughput screening of hundreds to thousands of candidate compounds, with only the most promising candidates being advanced to laboratory testing for further confirmation.

Moreover, in silico research can leverage publicly available biomolecular data (e.g., genomic, proteomic, and protein structural data) along with computational algorithms to predict molecules–target interactions with increasing accuracy (Carusi et al., 2021). One such application is *molecular docking,* a simulation technique used to predict the binding position and affinity of a ligand to a specific protein target (Makatita, 2020). Using this technique, researchers can preliminarily assess whether a candidate compound—such as curcumin—can interact with and inhibit the function of key pathogen proteins before to in vitro testing. With these advantages—cost-effectiveness, speed, and efficiency—in silico studies have become an integral part of the early stages of drug or biocontrol agent discovery. This includes efforts to identify inhibitory compounds for controlling *Ganoderma boninense*, the causal agent of basal stem rot in oil palm. Thus, the application of in silico methods is expected to accelerate the discovery of curcumin-based inhibitors from turmeric extracts, offering a promising strategy to mitigate CPO production losses in Indonesia more efficiently and effectively.

METHODS

The curcumin ligand (969516) was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), while the manganese peroxidase (1MNP), laccase (1KYA), and lignin peroxidase (1B85) proteins were obtained from RCSB PDB (https://www.rcsb.org/). All ligands and proteins were prepared using BIOVIA Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download) by removing water molecules and native ligands and adding polar hydrogen. Docking simulations were performed using PyRx software (https://pyrx.sourceforge.io/). Curcumin was inserted into the system using OpenBabel tools, while receptor proteins were inserted using the Load Molecule feature. All formats were converted from pdb to pdbqt so that they could be run using AutoDock Vina. The docking technique was performed using blind docking, which is running a docking simulation without first identifying the active site of the receptor protein by maximizing the grid box. The observed parameters included binding affinity, Root Mean Square Deviant (RMSD), bound amino acid residues, pocket site, ligand topography, ligand conformation position, and the depth of the active pocket where the ligand binds. The binding affinity and RMSD scores varied greatly depending on the device. Therefore, to prevent excessive variability, docking was performed three times using different devices. The docking results were analyzed using one-way ANOVA and followed by Tukey's test at a 95% confidence interval (α=0.05) using GraphPad Prism (https://www.graphpad.com/features). Next, the ligands resulting from the docking simulation were combined with the receptor protein using PyMol, and the interaction was analyzed using BIOVIA Discovery Studio software. However, this software was unable to identify the surface of the protein. Therefore, UCSF Chimera (https://www.rbvi.ucsf.edu/chimerax/) was used to revisualize the entire ligand-protein complex, and the layout was rearranged using InkScape (https://inkscape.id/).

RESULTS AND DISCUSSIONS

Curcumin Characterization in General

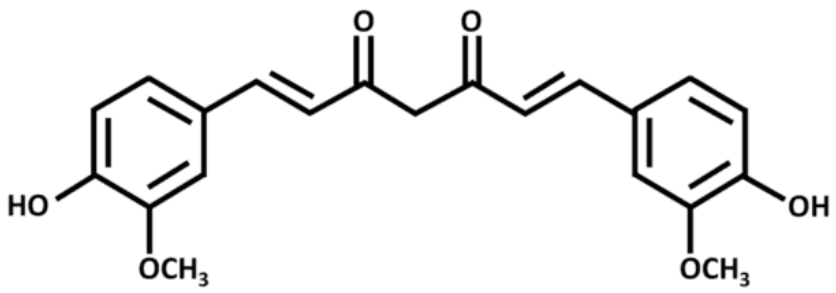

Figure 1. 2D structure of curcumin (Zhai et al., 2020)

Curcumin is the main polyphenolic compound found in the rhizome of *Curcuma longa* and is widely recognized for its important role in various biological applications (Amaroli et al., 2024). Structurally, curcumin consists of two aromatic rings, each substituted with hydroxyl (–OH) and methoxy ($–OCH_3$) groups, connected by an α,β-unsaturated linker containing a β-diketone moiety (Sharifi-Rad et al., 2020). The presence of phenolic and β-diketone groups allows curcumin to act as an electron donor, making it capable of forming stable coordination complexes with transition metal ions. In addition, the keto–enol tautomerism of curcumin provides structural flexibility, enhancing its ability to interact with both metal ions and biomolecules (Al-Noor et al., 2022).

In summary, the general characteristics of curcumin as a ligand are as follows: (1) it contains electron-donating functional groups (phenolic hydroxyl and β-diketone carbonyl) that allow coordination with metals, (2) it can switch between keto and enol forms, providing flexibility in binding, (3) it is relatively unstable in its free form, hence the need for metal complexation to enhance its chemical properties and biological activities, and (4) it is capable of forming stable complexes with various transition metal ions such as Cu^{2+}, Fe^{2+}/Fe^{3+}, and Zn^{2+}(Prasad, 2021), which are often associated with the enhanced biological activities of curcumin.

Curcumin interaction with MnP

Our molecular docking analysis has predicted curcumin occupies the active site of the manganese peroxidase (MnP) enzyme in a unique orientation resembling a "pinching" of the heme group, forming a interactions with key residues around the catalytic pocket such as Glu35, Glu39, and Asp179, and additional hydrophobic contacts with Phe269 and Leu169 (Figure 2). Similar binding behaviors of curcumin with heme-containing or oxidoreductase enzymes have been reported in recent docking and spectroscopic studies, supporting its ability to establish hydrogen bonds, hydrophobic contacts, and π–π stacking interactions within the active cavity (Li et al., 2024; Moon et al., 2024). Aromatic residues such as Phe269 allow π–π stacking with the aromatic ring of curcumin, while the hydrophobic residue Leu169 plays a role in strengthening the orientation of the ligand in the pocket. Analysis of the root means square deviation (RMSD) values, which range from 1.22 to 3.86 Å, provides insight into the stability of the ligand pose. RMSD values below 2 Å indicate a very stable bond orientation, while values above 3 Å, despite showing slight conformational variations, are still valid if the ligand maintains its presence at the active site. In this context, the RMSD of curcumin shows that the curcumin–MnP complex is relatively stable, despite reasonable pose diversity.

The chemical interactions between curcumin and MnP include hydrogen bonds via the phenolic hydroxyl or carbonyl group of curcumin with polar residues, hydrophobic interactions between the aromatic ring of curcumin and non-polar residues, and π–π stacking (Kostrzewa et al., 2021). The overall affinity of curcumin for manganese peroxidase (MnP) is primarily driven by hydrogen bonding, hydrophobic interactions, π–π stacking, and. Hydrogen bonds between curcumin's functional groups and polar residues in MnP, along with hydrophobic interactions between curcumin's aromatic rings and non-polar residues, provide stability in the active site (Li et al., 2024; Kostrzewa et al., 2021). Additionally, **π–π** stacking interactions between curcumin's aromatic rings and MnP's aromatic residues further stabilize the complex (Moon et al., 2024. These findings are consistent with recent studies emphasizing the multi-interaction mechanism of curcumin as the basis for curcumin's high affinity and biological potential as an antifungal agent (Chen *et al.*, 2018).

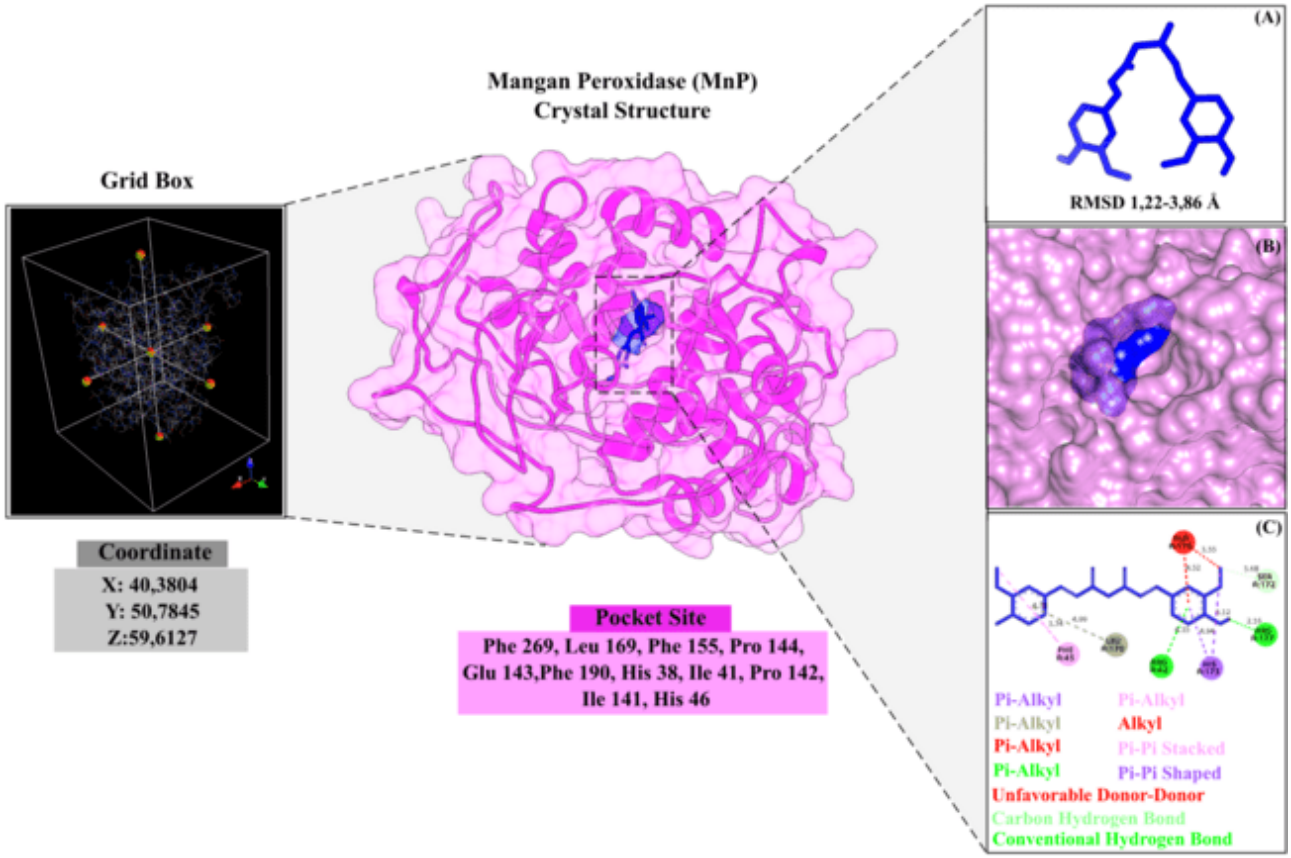

Figure 2. Molecular Docking Result on MnP Protein: (A) RMSD range, (B) visualization of active site, and (C) amino acids residues bound to ligand

Curcumin interaction with LiP

The molecular docking results show that curcumin is able to occupy the active site of the Lignin Peroxidase (LiP) domain B with a stable bond orientation (Figure 3). The RMSD values obtained were in the range of 1.472–2.49 Å, indicating that the curcumin pose was consistent and its conformation did not deviate far from the optimal position during the docking process (Moetlediwa et al., 2024). These values indicate that the curcumin–LiP complex is in the stable category. Therefore, curcumin is expected to have great potential as an active ligand or inhibitor against LiP protein. Curcumin adjusts itself in the active pocket with a curved conformation, allowing its two aromatic rings to interact with key residues on opposite sides of the active pocket. The aromatic residues Phe265 and Phe269 enable the formation of π–π stacking interactions with the aromatic rings of curcumin. In addition, polar residues such as Ser237 and Glu146 form carbon hydrogen bonds with the phenol and β-diketone groups of curcumin (Gupta *et al*., 2011). To our knowledge this specific residue-level interaction map has not been previously reported; however, curcumin is known to bind enzymes via hydrogen bonds, hydrophobic contacts, and π–π stacking, and LiP structures place multiple polar and aromatic residues in the active pocket (Sundaramoorthy et al., 2010). Hydrophobic interactions also dominate, especially with non-polar residues such as Val158, Leu169, Met172, and Ile154 that envelop the non-polar side of curcumin (Kurmi et al., 2025).

The combination of hydrogen bonds, hydrophobic interactions, and π–π stacking forms a multiple interaction system that strengthens curcumin's binding affinity to LiP. The π–π stacking occurs between the aromatic rings of curcumin and the aromatic residues in the active site of LiP, stabilizing the interaction (Moon et al., 2024). Additionally, curcumin forms hydrogen bonds with polar residues such as serine and glutamic acid, further enhancing the binding (Li et al., 2024). Hydrophobic interactions also play a role, as non-polar residues in the protein stabilize curcumin's position within the active site (Kostrzewa et al., 2021). These multiple interactions result in a strong and stable binding between curcumin and LiP, as confirmed by molecular docking analysis. This is also in line with recent studies showing that the more types of non-covalent interactions formed, the higher the stability of the resulting ligand–protein complex (Moon, 2024).

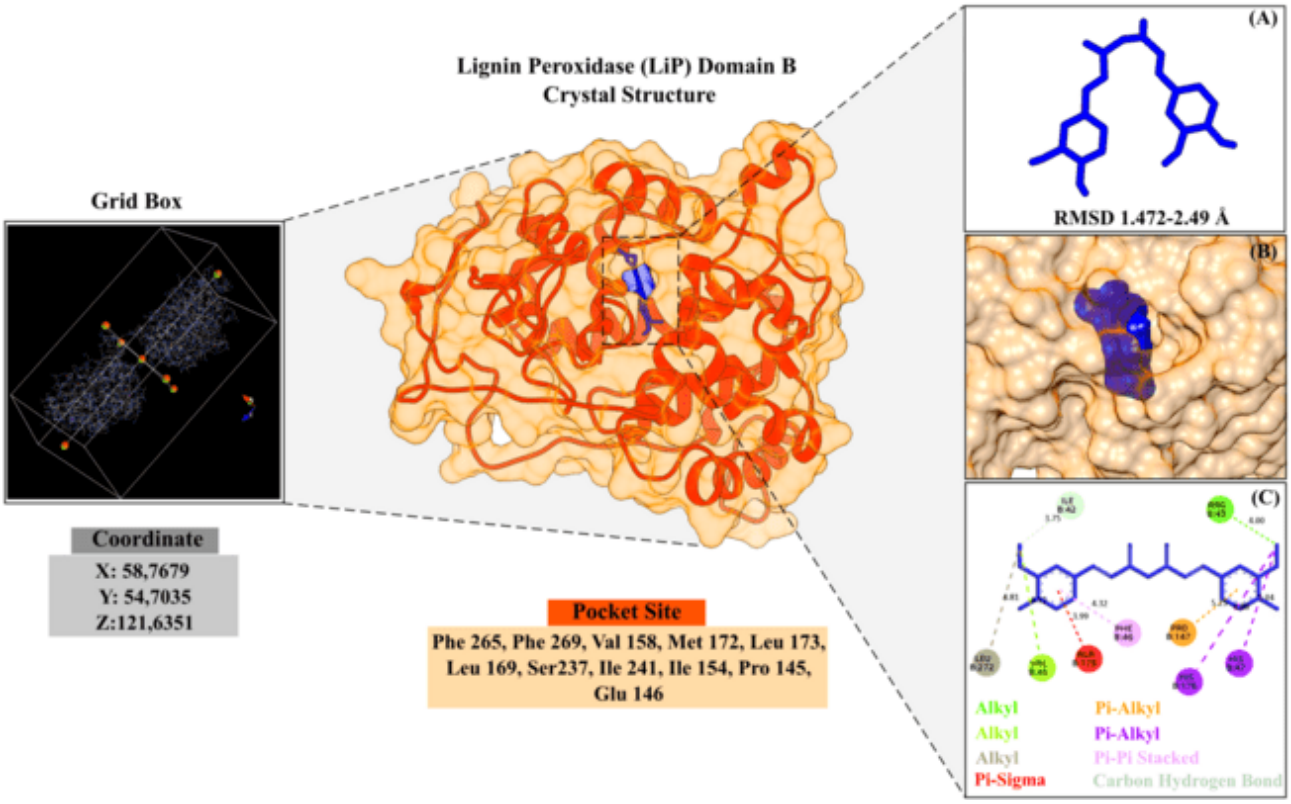

Figure 3. Molecular docking result on LiP: (A) RMSD range, (B) visualization of active site, and (C) amino acids residues bound to ligand

Curcumin interaction with Laccase

The docking results show that curcumin can occupy the active site of Laccase Domain C with a stable orientation and form a number of important interactions (Figure 4). The RMSD values obtained range from 2.76 to 4.46 Å, indicating some variation in the ligand pose within the active site, but still showing consistency in maintaining interactions with key residues. This RMSD value indicates that the orientation of curcumin is flexible enough to adjust its position without losing bond stability (Jack et al., 2024). Residues that play a role in the active pocket include Thr311, Pro307, Val309, Asn130, Asp128, Asn227, Asp101, Phe69, Ile226, Asp444, and Phe441. Curcumin is able to form hydrogen bonds with polar residues such as Thr311, Asn130, Asn227, Asp101, Asp128, and Asp444 through its phenolic and β-diketone groups (Anandaradje et al., 2024). In addition, the aromatic ring of curcumin interacts through π–π stacking with the aromatic residues Phe69 and Phe441, which helps increase the stability of the complex. Hydrophobic interactions with non-polar residues such as Pro307, Val309, and Ile226 also strengthen the orientation of the ligand within the pocket (Moon, 2024).

The combination of these various types of bonds creates a two-sided anchoring system, in which curcumin balances interactions with both polar and hydrophobic residues in the active pocket (Li et al., 2024). This pattern of multiple interactions is consistent with recent findings that curcumin and its derivatives are able to stabilize bonds with protein targets through multiple hydrogen bonds, π–π stacking, and hydrophobic contacts (Salamanova et al., 2024; Muñoz-Espinoza et al., 2025). Thus, it can be concluded that the curcumin–Laccase domain C complex is stable and has potential as a basis for the development of curcumin-based inhibitors.

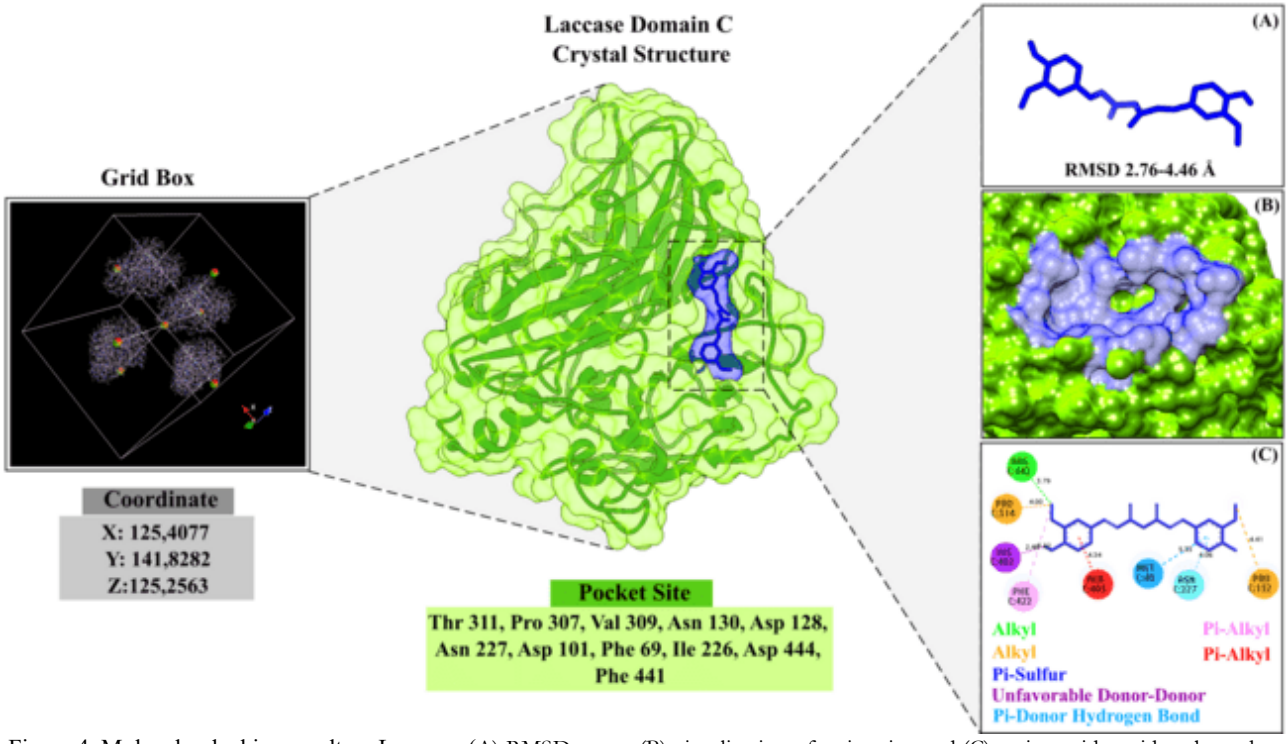

Figure 4. Molecular docking result on Laccase: (A) RMSD range, (B) visualization of active site, and (C) amino acids residues bound to ligand

Topography of the ligand–protein interaction

The ligand topography displays a range of color-coded visualizations across each parameter (Figure 5). Hydrogen bonds (H-bonds) (Figure 5A) show the key non-covalent interactions that support the ligand–protein complex. A study by Madushanka et al. (2023) confirms that hydrogen bonds play a major role in determining binding affinity and selectivity, where formed H-bonds can contribute approximately 1–4 kcal/mol of binding stabilization, depending on their geometric quality. Thus, the greater the number of H-bonds, whether donors or acceptors, the more stable the ligand–protein complex tends to be. Hydrogen bonds enhance ligand–protein stability only when the topography of the binding site allows them to become energetically meaningful. In a buried or well-shaped pocket, hydrogen bonds are shielded from solvent competition, adopt optimal geometry, and act as structural anchors that significantly lower the ligand's dissociation rate (Chen *et al.*, 2016). Based on Figure 2A, it can be seen that the interaction between curcumin and manganese peroxidase shows a more varied color distribution compared to the interaction between curcumin and either laccase or lignin peroxidase. This may contribute to the lower RMSD value of the curcumin-MnP complex.

Aromatic interactions (B) show bonds between two aromatic rings that provide stabilization (Budyak et al., 2013). Several types of aromatic interactions include π–π stacking, π–cation interactions, and edge-face interactions. π–π stacking occurs when two aromatic rings are arranged roughly parallel and close to each other, creating attractive forces via overlap of their π-electron clouds (Madushanka et al., 2023). A quantum computational study by Calinsky & Levy (2024) demonstrated that π–π stacking interactions can have binding energies comparable to hydrogen bonds, on the order of approximately –4 to –5 kcal/mol in solution. π–cation interactions occur between a positive charge from residues such as lysine or arginine and an aromatic ring (e.g., phenylalanine or tryptophan). π–cation interactions are driven by electrostatic attraction between the cation and the aromatic electron cloud and are regarded as among the strongest non-covalent interactions in chemical biology (Park et al., 2023). The edge-face or CH–π interaction involves a hydrogen at the edge of one ring interacting with the face of another ring, this type generally contributes more weakly than other aromatic interactions (Jennings et al., 2013). Nevertheless, their high frequency and number in protein structures can make their cumulative contribution non-negligible (Calinsky & Levy, 2024). The ligand topography for LiP binding is dominated by edge-face interactions compared with laccase and MnP.

Interpolated charge (C) depicts the electrostatic charge distribution on the molecular contact surface that influences molecular recognition and the strength of complex interactions (Di Savino et al., 2021). Soler et al. (2025) revealed that electrostatically complementary surfaces increase affinity through the formation of strong

ionic bonds or salt bridges; however, charge distribution must be complementary on both sides. In the figure, blue indicates positive charge while red indicates negative charge. Laccase appears mainly from white to light pink, indicating a relatively neutral to slightly acidic environment. This means curcumin, which has phenolic –OH groups, may not have many positive charges available for strong ionic interactions. MnP and LiP are both paler and more neutral in color compared with laccase—showing only small areas of blue/red—indicating minimal electrostatic interactions and therefore that curcumin binding relies more on hydrophobic and π interactions. This is in line with research conducted by Xia et al (2018) in which they marked the surface of proteases with a positive charge in blue, while negative charges were marked in red.

Hydrophobic interactions (D) are a primary driving force for binding affinity in many complexes (Lindman et al., 2021). The hydrophobic effect arises from water's tendency to exclude non-polar surfaces (Sun, 2022). When a ligand occupies a hydrophobic pocket, structured water molecules that were "trapped" in that site are released into the bulk solvent (Eberhardt & Forli, 2023). Mahmud et al. (2023) emphasize that this release of water provides a large entropy gain to the system, which pulls ligand and protein closer together and reduces free energy. Blue indicates hydrophilic regions, while brown indicates hydrophobic regions. In laccase, the surface shows a mix of blue with some brown, indicating that curcumin's hydrophobic interactions with the protein are weak, while MnP and LiP display more prominent brown spots. According to Weiß et al. (2018), water reorganization near hydrophobic surfaces creates directional forces that drive ligand orientation and specificity. This suggests that MnP and LiP-curcumin complexes have a more locked orientation and reduced conformational flexibility, making them more stable (Agosta & Cozzini, 2024) as indicated by their lower RMSD ranges compared to the curcumin-laccase complex. Ionization and protonation properties (F) describe the acid–base characteristics of ligand functional groups relative to active-site amino acid residues. For example, negatively charged aspartate/glutamate residues and positively charged lysine/arginine residues (Ghatas et al., 2025). Protonation state is often required to form optimal interactions. For example, a ligand bearing an amine group must be protonated (positively charged) to bind strongly to negatively charged residues (e.g., aspartate/glutamate) via ionic interactions (Vatheuer et al., 2025). Blue spots indicate basic regions, while red spots indicate acidic regions. The laccase complex shows a few red spots, indicating surrounding residues tend toward acidic character (possibly aspartate or glutamate)—however, because curcumin does not carry a positive charge, ionic interactions remain limited. The MnP complex exhibits more blue exposure, which can support hydrogen bonding with curcumin's phenolic –OH groups. The LiP complex shows a dominant pale-blue coloration, indicating a basic environment that favors stable hydrogen-bonding interactions with curcumin.

Solvent Accessible Surface (SAS) (E) refers to how much of a molecule's surface is exposed to solvent (Tien et al., 2013). In a ligand–protein complex, the area of ligand or protein surface that becomes "buried" upon binding correlates positively with affinity because a larger interface allows for more points of contact, such as van der Waals forces, hydrogen bonds, and hydrophobic interactions, to form between the binding partners, contributing to greater binding energy and affinity. (Chen et al., 2013). Blue spots indicate solvent-exposed regions, while green spots denote buried, non-solvent-exposed regions (Elamin et al., 2022). The laccase, MnP, and LiP complexes all showed a large number of blue spots, indicating that curcumin remained sufficiently exposed to the solvent in all three complexes, which should have resulted in weaker binding affinity and higher RMSD ranges. However, the molecular docking results showed contrasting results, suggesting that there may be other factors that caused SAS to have no significant effect on the results.

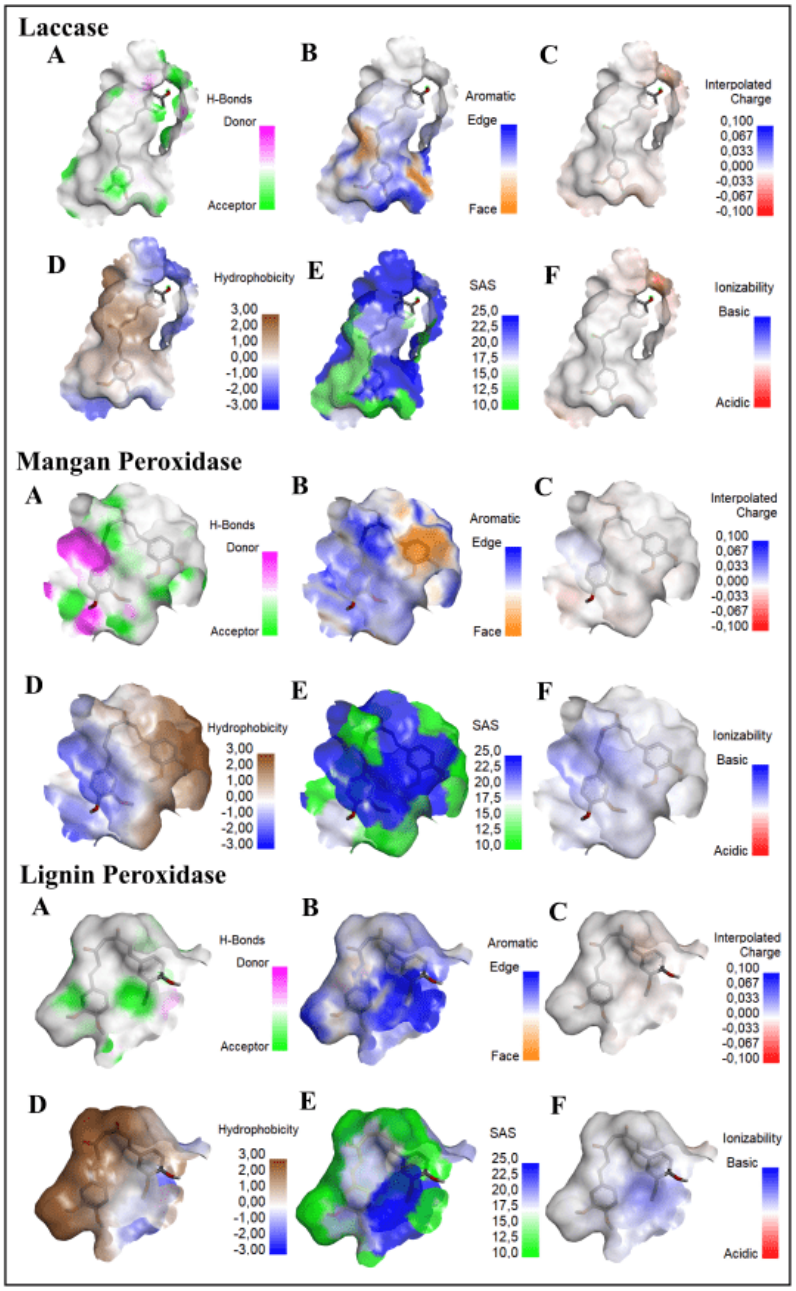

Figure 5. Ligand–receptor topography: (A) hydrogen bonding, (B) aromatic interactions, (C) electrostatic charge distribution, (D) hydrophobicity, (E) solvent-accessible surface (SAS), and (F) ionization/protonation properties.

Data Analysis Result on Curcumin Interaction with Protein Receptor

Based on the statistical analysis (Figure 6A), the curcumin–laccase complex displays the least negative value (−6.53 kcal/mol), whereas curcumin–MnP and curcumin–LiP show more negative affinities (around −9.0 kcal/mol). In molecular docking, a more negative binding affinity indicates a stronger and more thermodynamically favorable interaction. Therefore, numerically, curcumin binds weaklier to laccase compared with MnP and LiP (P_{Value} 0.0003 < P_{table} 0.001). The significance of the difference in interaction strength between the three is most likely due to the location of ligand binding to the receptor protein and the residues. In MnP and LiP, curcumin is located inside the active pocket or cavity of the protein, providing a closed environment that allows for more molecular contact and reduces ligand exposure to the solvent. Meanwhile, curcumin in laccase only attaches to the surface. This is in line with Zhao et al. (2020), who stated that high affinity between ligands and proteins can only be obtained if the contact area between the ligand and the protein is sufficiently large. Curcumin bound to the surface of laccase will more

easily compete with water molecules, thereby reducing molecular contact, which leads to weakened interactions. In addition, the exposure of curcumin to solvent on the surface of laccase increases entropic cost and reduces enthalpy which reduces the strength of the interaction (Galano-Frutos et al., 2015; Matricon et al., 2021). In contrast, the cavities of MnP and LiP proteins ligands are positioned in such a way that they are strongly bound through various non-covalent interactions and protected from solvent interference.

Research conducted by Guo et al. (2015) obtained statistical data showing that ~97% of ligands in the "strong interaction" category in crystallographic protein complexes were found to be bound to the deepest and largest protein pockets. Not only do deep active cavities expand molecular contact, but they are also generally more hydrophobic than surface sites. The more hydrophobic interactions possible, the relatively stronger the interaction between the ligand and protein, along with a more optimal oriented position. A study conducted by Ali et al. (2023) showed that mutation of the aspartic acid (Asp205) amino acid in laccase drastically reduced binding ability/activity because the ligand lost hydrogen anchors and important electrostatic interactions. The lower and upper RMSD values did not show any significant differences between groups with $P_{Value} < 0.05$ (The p-value in the figure is not shown because it is not significantly different), indicating that the conformation of curcumin against the three receptor proteins was relatively consistent (Figure 6. B & C)

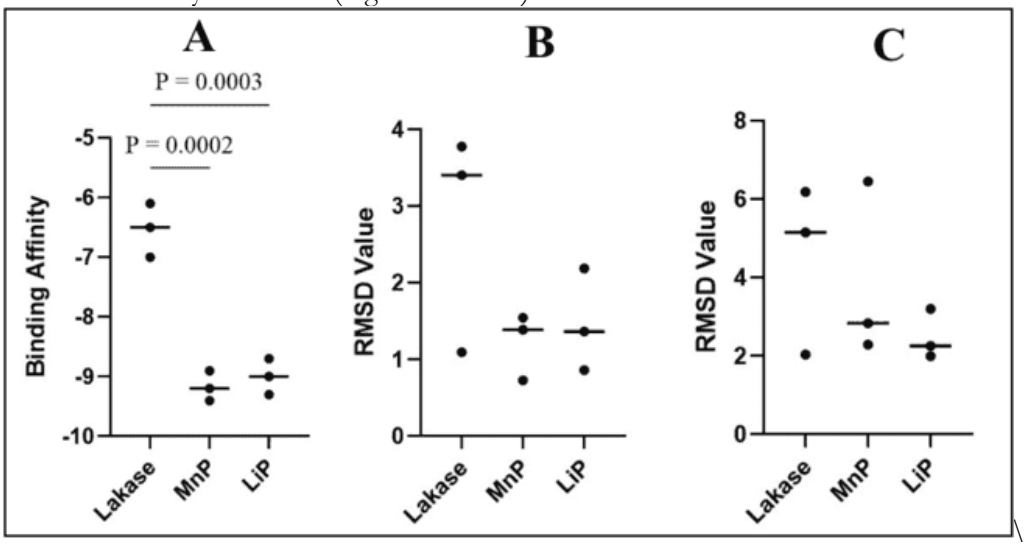

Figure 6. Results of statistical tests of the interaction between curcumin and receptor proteins: A) binding affinity, B) lower RMSD, and C) upper RMSD

CONCLUSIONS

The present study demonstrates the effectiveness of in silico approaches in analyzing the pathogenic fungus *Ganoderma boninense*, the causal agent of basal stem rot (BSR) that threatens oil palm production in Indonesia. By employing bioinformatics tools and molecular docking simulations, we were able to characterize the structural and functional properties of key ligninolytic enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase, which play a central role in fungal virulence and degradation of host plant tissues. The docking analysis revealed that curcumin, a bioactive compound derived from natural sources, exhibited favorable binding affinity and multiple stabilizing interactions with these enzymes, suggesting its potential as an eco-friendly antifungal candidate.

Beyond simply identifying potential inhibitors, the use of in silico methods in this research proved advantageous because it allows rapid, cost-efficient, and high-throughput screening of ligand–protein interactions before moving into more resource-intensive laboratory experiments. This approach not only saves time and resources but also provides detailed insights into the molecular mechanisms that underlie host–pathogen interactions. Importantly, the computational results obtained here provide a rational foundation for subsequent experimental validation, such as in vitro inhibition assays or in planta studies, to confirm the antifungal potential of curcumin against *G. boninense*.

Nevertheless, it should be noted that in silico predictions are inherently limited by the accuracy of protein models, docking algorithms, and scoring functions. Binding affinities observed in simulations may not fully replicate the dynamic complexity of biological systems, and factors such as bioavailability, stability, and host metabolism must be evaluated through experimental studies.

Taken together, these findings highlight the value of computational biology as a powerful complementary strategy in plant pathology research. In the context of Indonesia's palm oil industry, where BSR poses a significant

economic and agricultural challenge, in silico analyses can accelerate the discovery of sustainable disease management strategies. The integration of natural bioactive compounds and modern computational methods offers a promising path forward toward environmentally friendly and effective solutions for protecting oil palm plantations from *Ganoderma* infection.

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