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# Lansium domesticum metabolites interfere with digestive enzyme function in the rice weevil sitophilus oryzae

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ABSTRACT

Pectin methylesterase (PME) plays a key role in polysaccharide digestion in the rice weevil *Sitophilus oryzae*, making it a potential molecular target for pest control. This study employed structure-based *in silico* analysis to evaluate selected metabolites of *Lansium domesticum* as PME inhibitors. Representative compounds were chosen according to predicted interference mechanisms and docked into the validated catalytic site. All complexes showed structural stability, while interaction patterns revealed six inhibition modes: competitive mimicry, catalytic disruption, orientation interference, channel blocking, polymer mimicry, and interfacial modulation, whereas polyphenols and glycosides produced the strongest interference. The findings indicate that *L. domesticum* metabolites can disrupt digestive enzymatic processes through multiple complementary pathways, supporting their potential development as biologically derived pest management agents.



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## INTRODUCTION

Post-harvest losses caused by insects remain a major constraint on food security, particularly in tropical regions where environmental conditions favor rapid pest proliferation. One of the most destructive primary pests of stored cereals is the rice weevil (*Sitophilus oryzae*), which infests intact grains and completes its life cycle inside the kernel (Berhe et al., 2022; Awadallah et al., 2024; Roy et al., 2024). Feeding by larvae and adults leads to weight reduction, nutritional deterioration, and decreased germination capacity (Singh & Sharma, 2024; Awadallah et al., 2024; Roy et al., 2024). Control strategies largely depend on fumigants and synthetic insecticides; however, extensive use has resulted in resistance development, environmental contamination, and safety concerns for consumers and storage workers (Nayak et al., 2020; Paul et al., 2020). These limitations have

driven increasing interest in biologically based pest management approaches that disrupt insect physiology rather than induce acute toxicity.

Targeting digestive processes represents a promising alternative strategy. Stored-grain insects rely on enzymatic degradation of plant structural polysaccharides to obtain nutrients. Pectin methylesterase (PME) plays a major role in this process by catalyzing the demethylation of pectin, a major component of plant cell walls (Wu et al., 2018; Kumar et al., 2023). The reaction modifies cell wall integrity and facilitates subsequent hydrolysis by other enzymes (Wormit & Usadel, 2018; Kumar et al., 2023). Interfering with PME activity therefore reduces nutrient accessibility and indirectly suppresses insect development (Reem et al., 2020). Compared with neurotoxic insecticides, inhibition of digestive enzymes is considered more selective and environmentally compatible.

Plants produce diverse secondary metabolites capable of interacting with proteins through hydrogen bonding, hydrophobic contacts, electrostatic attraction, and steric obstruction (Shahidi & Dissanayaka, 2023). Unlike single-target synthetic insecticides, phytochemicals often affect enzymes through multiple physicochemical mechanisms, including substrate mimicry, catalytic interference, and blockage of substrate access pathways (Suganya et al., 2022; Zai et al., 2025). Such mechanistic diversity may lower the probability of resistance development because inhibition does not rely on a single biochemical pathway (Kaur et al., 2022; Suganya e al., 2022). Structure-based computational approaches provide an effective preliminary method to predict interactions and prioritize compounds for further validation (Oktariansyah et al., 2025; Jung et al., 2025).

*Lansium domesticum* (*duku* or *langsat*) is a tropical fruit species widely distributed in Southeast Asia, especially Indonesia, and traditionally associated with insect-repellent properties, particularly in its peel and seeds (Yamin et al., 2020; Abdallah et al., 2022). Phytochemical studies have identified numerous triterpenoids, phenolic compounds, limonoids, and glycosides from this plant, several of which exhibit insecticidal or antifeedant activity (Mayanti et al., 2022; Rudiyansyah et al., 2025). Despite these findings, the molecular basis of its bioactivity for pest biological control remains unclear, as targeting digestive enzymes and how they interact structurally with such targets has not been systematically explored.

Recent developments in molecular modeling allow enzyme inhibition to be interpreted beyond simple binding affinity comparisons. Compounds with similar docking energies may exert entirely different biological effects depending on their spatial orientation and interaction type within the catalytic environment (Tateing & Suree, 2022; Castillo-Campos et al., 2023; Pesaresi et al., 2023). Small polar molecules may mimic natural substrates, planar aromatic compounds may disturb substrate orientation, and bulky amphipathic molecules may block access channels required for macromolecular substrates (Itskanov et al., 2022; Hendrickson-Rebizant et al., 2024; Çetin et al., 2025).

In this context, PME from *S. oryzae* represents a suitable molecular target for evaluating plant-derived inhibitors intended for biological control. A structure-based *in silico* analysis enables mapping of ligand interaction with catalytic residues, binding grooves, and substrate pathways (Nawaz et al., 2025; Oktariansyah et al., 2025). By comparing compounds representing different physicochemical categories, it becomes possible to identify diverse inhibitory behaviors within a single plant metabolome.

The present study investigates selected metabolites of *L. domesticum* against *S. oryzae*'s PME using a structure-based computational approach. Rather than focusing solely on docking scores, the analysis emphasizes interaction patterns, spatial positioning, and structural consequences to classify modes of inhibition. This work aims to demonstrate that plant secondary metabolites can interfere with insect digestive enzymes through multiple mechanistic pathways, providing a conceptual foundation for enzyme-targeted biological control of stored-grain pests.

## RESEARCH METHODS

### Research Design

This study employed a structure-based computational design to evaluate potential inhibition mechanisms of pectin methylesterase (PME) in the rice weevil (*S. oryzae*) by secondary metabolites of *L. domesticum*. The workflow emphasized mechanistic interpretation rather than affinity-based virtual screening. The research was conducted from January to February 2026 at the Genetics Laboratory, Sriwijaya University. The method is modified from Oktariansyah et al. (2025) and Rosell & Fernández-Recio (2020) by integrating docking interactions with spatial and stability assessment, the study translated molecular binding behavior into biologically interpretable modes of enzyme interference relevant to *S. oryzae* control.

### Instruments

Computational analyses were performed using a personal workstation running the Windows operating system. Protein structure preparation, cavity detection, interaction visualization, and post-docking structural refinement were conducted using BIOVIA Discovery Studio (BIOVIA Dassault Systèmes, 2021). Molecular docking simulations were performed using AutoDock Vina (Trott & Olson, 2010) implemented in PyRx (Dallakyan & Olson, 2015), which was also used for ligand format conversion and preliminary geometry stabilization through OpenBabel integration. Reconstruction and merging of protein–ligand complexes generated from docking were carried out using PyMOL (Schrödinger LLC, 2015) to ensure correct spatial positioning before interaction analysis. 3D molecular structures of ligands were obtained from public chemical databases, including PubChem, and processed using OpenBabel within PyRx. Structural stability evaluation through root mean square deviation (RMSD) calculations and interaction mapping was performed within the Discovery Studio environment. All visualization figures, including 3D binding poses and 2D interaction diagrams, were generated using BIOVIA Discovery Studio for structural interpretation and presentation.

### Procedures

#### Protein Structure Preparation

The 3D of Pectin methylesterase (PME; PDB ID: 4PMH) was retrieved from the Protein Data Bank (Berman et al., 2000) and processed in BIOVIA Discovery Studio. Crystallographic water molecules, buffer components, and non-structural heteroatoms were removed to avoid artificial electrostatic bias during docking calculations. Removal of crystallographic solvent molecules is recommended when solvent participation in catalysis is not experimentally validated and when comparative docking is performed (Pagadala et al., 2017). No manual protonation adjustment or external energy minimization was applied. The receptor was intentionally preserved in its experimental crystallographic conformation and treated as rigid throughout the simulations to prevent artificial conformational drift and maintain catalytic geometry during comparative docking analysis (Pinzi & Rastelli, 2019).

#### Ligand Preparation

Ligands consisted of galacturonic acid (native substrate), selected secondary metabolites of *L. domesticum* (Abdallah et al., 2022), and a reference compound. Molecular structures were obtained from public chemical databases (https://pubchem.ncbi.nlm.nih.gov/) and converted into docking-compatible formats using PyRx (Dallakyan & Olson, 2015) with OpenBabel integration (O'Boyle et al., 2011). Each ligand underwent preliminary energy minimization to remove steric strain originating from database coordinates. This stabilization step prevents unrealistic docking conformations while avoiding bias introduced by extensive pre-optimization (Ferreira et al., 2019). Hydrogen atoms and bond orders were subsequently assigned automatically in Discovery Studio,

and final energetic treatment was deferred to docking and refinement stages to ensure consistent force-field handling among ligands (Pagadala et al., 2017).

### Mechanism-Oriented Representative Ligand Selection

A dataset of 154 reported secondary metabolites of *L. domesticum* was compiled from phytochemical literature (Table S1. Appendix). Instead of exhaustive virtual screening, representative compounds were selected based on predicted interference mechanisms toward carbohydrate-active enzymes. Selection considered physicochemical determinants known to influence ligand accommodation in enzyme binding grooves, including molecular size, polarity, aromaticity, rigidity, and amphipathicity (Samaei-Daryan et al., 2017; Olubiyi et al., 2017). Compounds were grouped into mechanistic archetypes representing substrate mimicry, aromatic stacking interference, catalytic perturbation, channel blocking, surface adsorption, non-specific hydrophobic occupancy, polymeric substrate analogues, and interfacial modulation. Mechanism-driven selection is recommended to preserve functional diversity while reducing redundancy in targeted docking studies (Pinzi & Rastelli, 2019).

### Active Site Determination

Because the PME structure lacks a co-crystallized ligand, the active site was determined through functional validation. Putative binding cavities predicted in Discovery Studio were individually evaluated by docking galacturonic acid. The cavity interacting with catalytic residues was defined as the functional active site. Substrate-guided active-site identification improves docking reliability when experimental ligand coordinates are unavailable (Pagadala et al., 2017; Ferreira et al., 2019).

### Molecular Docking

Docking simulations were performed using AutoDock Vina implemented in PyRx. The receptor was treated as rigid and ligands as flexible to enable comparative binding behavior analysis rather than induced-fit modeling (Forli et al., 2016; Pinzi & Rastelli, 2019). Multiple binding poses were generated and ranked according to predicted binding affinity. The resulting complexes were reconstructed using PyMOL for structural interpretation.

### Data Analysis

The reconstructed complexes were relaxed in Discovery Studio using the CHARMm force field with Momany–Rone partial charges (Brooks et al., 2009; Momany & Rone, 1992). Local relaxation after docking reduces steric clashes and improves interaction realism without altering global binding orientation (Ferreira et al., 2019). Non-covalent interactions were analyzed, including hydrogen bonding, electrostatic contacts, and hydrophobic/π interactions. Mapping residue-level interactions is necessary to interpret enzyme inhibition mechanisms beyond affinity scores alone (Zhang et al., 2023). Complex stability was evaluated using root mean square deviation (RMSD). Low RMSD values indicate localized binding perturbation without global structural change, a typical interpretation in structure-based inhibitor analysis (Moussaoui et al., 2024). Interaction patterns and spatial positioning were integrated to infer mechanisms of action, classifying ligands into substrate-like binders, channel blockers, catalytic perturbators, interfacial modulators, or non-specific binders. Docking outputs were analyzed hierarchically. All ligands were first reported for transparency, after which ligands producing equivalent spatial and functional outcomes were consolidated into mechanistic categories. Functional grouping of docking results is recommended to translate computational binding data into biologically testable hypotheses (Yang et al., 2022).

## RESULTS

### Energetic Evaluation and Structural Stability of PME–Ligand Complexes

Molecular docking generated stable complexes between pectin methylesterase (PME) and all tested ligands (Table 1).

**Table 1.** Binding Energetics and Structural Stability of PME–Ligand Complexes

| Ligand | Chemical Group | Binding Affinity (kcal/mol) | RMSD Cα (Å) | RMSD Backbone (Å) | RMSD All Atom (Å) | Structural Interpretation |
|---|---|---|---|---|---|---|
| Galacturonic Acid | Native substrate | -5.8 | 0.000 | 0.055 | 0.000 | Stable productive complex |
| Lansic Acid | Triterpenoid | -5.8 | 0.000 | 0.055 | 0.000 | Stable non-productive binding |
| Imidacloprid | Insecticide | -6.0 | 0.000 | 0.055 | 0.000 | Peripheral association |
| Ellagic Acid | Polyphenol | -7.5 | 0.000 | 0.055 | 0.000 | Strong catalytic interaction |
| Quercetin | Flavonoid | -7.1 | 0.000 | 0.055 | 0.000 | Stable peripheral binding |
| Gallic Acid | Phenolic acid | -5.4 | 0.000 | 0.055 | 0.000 | Weak catalytic mimic |
| Palmitoleic Acid | Lipid | -4.2 | 0.000 | 0.055 | 0.000 | Surface association |
| τ-Muurolol | Terpenoid | -6.1 | 0.000 | 0.055 | 0.000 | Hydrophobic occupancy |
| Citric Acid | Organic acid | -5.7 | 0.000 | 0.055 | 0.000 | Non-productive catalytic binding |
| Catechin | Flavonoid | -7.1 | 0.000 | 0.055 | 0.000 | Aromatic groove binding |
| Rutin | Glycoside | -8.1 | 0.000 | 0.055 | 0.000 | Extended groove occupation |
| Lansioside A | Glycoside | -7.2 | 0.000 | 0.055 | 0.000 | Surface occlusion |
| Digitoxigenin | Sterol | -7.3 | 0.000 | 0.055 | 0.000 | Interfacial stabilization |
| β-Sitosterol | Sterol | -6.9 | 0.000 | 0.055 | 0.000 | Surface interference |

Binding affinity values ranged from −4.2 to −8.1 kcal/mol, indicating energetically feasible associations for both natural substrate and secondary metabolites. The strongest predicted binding was observed for rutin (−8.1 kcal/mol), followed by ellagic acid (−7.5 kcal/mol), Digitoxigenin (−7.3 kcal/mol), and Catechin (−7.1 kcal/mol), whereas palmitoleic acid displayed the weakest interaction (−4.2 kcal/mol). Despite variability in affinity, structural deviation remained negligible across all complexes. The Cα RMSD was 0.000 Å, and backbone RMSD remained constant at 0.055 Å for every ligand. Whole-protein RMSD also remained zero after refinement, indicating the absence of global conformational changes. These values demonstrate that ligand binding produced local interaction adjustments rather than structural distortion of the enzyme.

The uniform structural stability suggests that the differences observed among ligands are primarily determined by interaction geometry and binding location rather than methodological variation in docking. Consequently, biological interpretation can focus on spatial interaction patterns rather than binding energy alone. Notably, ligands with similar binding energies exhibited different structural interpretations. For example, galacturonic acid and lansic acid shared identical affinity (−5.8 kcal/mol), yet the former formed a productive catalytic complex while the latter produced non-productive binding. This indicates that binding affinity alone cannot predict enzymatic outcome and must be interpreted together with interaction topology.

### Residue-Level Interaction Patterns Reveal Distinct Binding Regions

Analysis of residue contacts demonstrated that ligands occupy multiple functional regions within PME rather than a single universal binding pocket (Table 2). After confirming docking stability, residue-level interactions were analyzed. Not all ligands were displayed in Table 2 because many compounds shared identical binding regions and interaction motifs. To avoid redundancy, ligands showing equivalent residue contacts were grouped and represented by structurally informative examples. The selected ligands represent each unique binding topology observed within the full dataset.

**Table 2.** Key Interacting Residues and Dominant Molecular Interaction Types

| Ligand | Main Interacting Residues | Distance Range (Å) | Dominant Interaction | H-bond | π | Electrostatic | Binding Region |
|---|---|---|---|---|---|---|---|
| Galacturonic Acid | GLY205, GLY207, TYR203, ASP258, GLY231 | 1.77–2.82 | Polar catalytic | 5 | 0 | 0 | Catalytic cleft |
| Lansic Acid | ARG290, TRP292, PHE229, GLY295, VAL296, ASP293 | 2.33–5.30 | Hydrophobic anchor | 6 | 7 | 0 | Entrance channel |
| Ellagic Acid | GLY205, SER206, THR306, ASP258, ASP260, PRO261, | 2.13–5.44 | π–anion catalytic | 4 | 2 | 3 | Catalytic vicinity |

| Ligand | Main Interacting Residues | Distance Range (Å) | Dominant Interaction | H-bond | π | Electrostatic | Binding Region |
|---|---|---|---|---|---|---|---|
| | PRO308 | | | | | | |
| Quercetin | SER209, LYS263, GLY231, SER294, ASP260, PRO308 | 2.23–4.68 | Aromatic–polar | 2 | 2 | 1 | Peripheral site |
| Catechin | THR333, TRP292 | 2.41–5.06 | π-stacking | 2 | 4 | 0 | Aromatic clamp |
| Rutin | GLN199, ASP200, ASP226, TRP292, VAL296, THR333 | 1.87–5.22 | Distributed network | 9 | 2 | 1 | Binding groove |
| Lansioside A | ASN192, ARG194, ASN218, TYR220, GLU222, SER238, THR240, TYR242, PRO98 | 1.78–5.47 | Surface network | 4 | 8 | 0 | Extended groove |
| Digitoxigenin | ASP226, SER297, TRP292 | 2.09–5.46 | Hydrophobic interface | 1 | 5 | 0 | Surface interface |
| β-Sitosterol | ASN192, ALA193, ARG194, TYR220, TYR242 | 2.41–5.38 | Hydrophobic interface | 1 | 7 | 0 | Surface interface |

Note: π = Hydrophobic

The native substrate galacturonic acid bound inside the catalytic cleft involving GLY205, GLY207, TYR203, ASP258, and GLY231. The interaction distances (1.77–2.82 Å) and the presence of five hydrogen bonds without hydrophobic or electrostatic contributions indicate a classical polar catalytic recognition pattern typical for carbohydrate-active enzymes. This configuration corresponds to a productive catalytic binding geometry. In contrast, the triterpenoid lansic acid interacted mainly with ARG290, TRP292, PHE229, GLY295, VAL296, and ASP293 at longer distances (2.33–5.30 Å). The interaction network was dominated by hydrophobic and π contacts accompanied by several hydrogen bonds. Because these residues are located at the entrance channel, the ligand formed a hydrophobic anchoring barrier rather than catalytic binding.

Ellagic acid showed a mixed interaction pattern near the catalytic center. Contacts with GLY205, SER206, THR306, ASP258, and ASP260 occurred within 2.13–5.44 Å and involved hydrogen bonds, π interactions, and electrostatic contacts. The presence of electrostatic

contributions together with aromatic interactions suggests π–anion stabilization around catalytic residues, indicating catalytic perturbation rather than substrate mimicry. Flavonoids displayed peripheral recognition modes.

Quercetin interacted with SER209, LYS263, GLY231, SER294, ASP260, and PRO308 through weak hydrogen bonding and limited electrostatic contact, indicating aromatic–polar association away from the catalytic core. Catechin formed π-stacking interactions primarily with TRP292 and THR333, showing dominance of hydrophobic stacking interactions rather than direct catalytic engagement. Large glycosides occupied extended grooves. Rutin formed nine hydrogen bonds across GLN199, ASP200, ASP226, TRP292, VAL296, and THR333, producing a distributed network spanning 1.87–5.22 Å. Lansioside A interacted with numerous surface residues, including ASN192, ARG194, TYR220, and THR240, combining hydrogen bonding and hydrophobic contacts across a broader spatial range (1.78–5.47 Å). These patterns indicate extended groove occupation rather than localized binding.

Sterols interacted primarily through hydrophobic interfaces. Digitoxigenin and β-sitosterol associated with surface residues such as TRP292, TYR220, and ARG194, forming minimal hydrogen bonds but multiple hydrophobic contacts across distances above 2 Å. The absence of catalytic contacts suggests interfacial association rather than direct enzymatic inhibition.

Overall, the interaction mapping demonstrates that PME ligands segregate into catalytic cleft binders, channel blockers, peripheral aromatic binders, groove-occupying glycosides, and surface hydrophobic modulators, providing structural evidence for multiple functional interference mechanisms.

## Mechanistic Classification of PME Interference

Once distinct binding regions were identified, ligands were further consolidated according to functional consequence rather than chemical identity. Table 3, therefore, presents mechanistic classes instead of individual molecules. Multiple ligands belonged to each class, indicating that inhibition behavior is governed by spatial interaction topology rather than specific chemical structure. The reduction in ligand number at this stage represents biological generalization, not selective reporting.

**Table 3.** Mechanistic Classification of PME Inhibition Modes

| Mechanistic Class | Representative Ligands | Mode of Interference | Prediction Effect on PME Activity |
|---|---|---|---|
| Productive substrate | Galacturonic acid | Correct catalytic positioning | Normal catalysis |
| Competitive mimics | Gallic acid, Citric acid | Occupy catalytic pocket | Reduced efficiency |
| Catalytic disruptors | Ellagic acid | Disturbs catalytic chemistry | Reaction failure |
| Orientation disruptors | Quercetin, Catechin | Misalign polysaccharide | Incorrect processing |
| Channel blockers | Lansic acid | Prevent substrate entry | No access |
| Polymer mimics | Rutin, Lansioside A | False substrate binding | Enzyme occupied |
| Interfacial sterols | Digitoxigenin, β-Sitosterol | Disturb substrate approach | Poor processing |
| Non-specific occupants | Palmitoleic Acid, τ-Muurolol | Passive association | Negligible |

The native substrate galacturonic acid maintained proper catalytic orientation, supporting normal enzymatic activity. Small organic acids acted as competitive mimics by occupying the catalytic pocket but failing to undergo productive turnover, thereby reducing catalytic efficiency. Ellagic acid functioned as a catalytic disruptor by directly perturbing catalytic residues, preventing reaction progression. Flavonoids e.g., Quercetin, Catechin, acted as orientation disruptors, misaligning the polysaccharide substrate through aromatic stacking interactions. Lansic acid operated as a channel blocker, physically preventing substrate entry into the catalytic cleft. Glycosides e.g., Rutin, Lansioside A, acted as polymer mimics by occupying the binding groove and trapping the enzyme in a false-substrate state. Sterols, including Digitoxigenin and β-Sitosterol, functioned as interfacial modulators, altering substrate approach without occupying the catalytic center. Lipids (Palmitoleic Acid) and terpenoids (τ-Muurolol) produced only passive hydrophobic association and negligible inhibition. This classification reveals that PME inhibition by plant metabolites is multi-mechanistic rather than affinity-dependent. Different chemical architectures target different structural vulnerabilities of the enzyme.

## Structural Visualization of Representative Binding Modes

Because ligands within each mechanistic class shared nearly identical binding geometry, only representative complexes were visualized in Figures 1 – 5. Visual inspection confirmed catalytic alignment for galacturonic acid, catalytic disruption by ellagic acid, channel occlusion by lansic acid, groove occupation by rutin, and surface modulation by sterols. These representative models illustrate recurring spatial behaviors observed across multiple compounds.

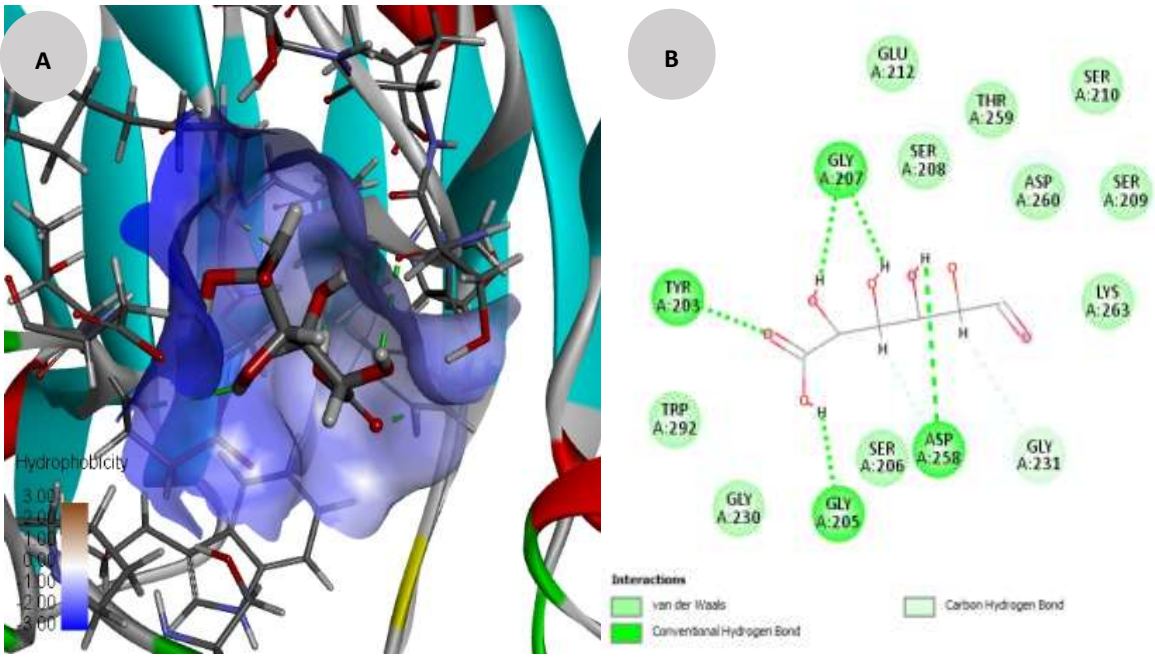


**Figure 1.** (A) Docking Poses and (B) Interaction Maps of Galacturonic Acid (Key observation: Correct catalytic orientation)

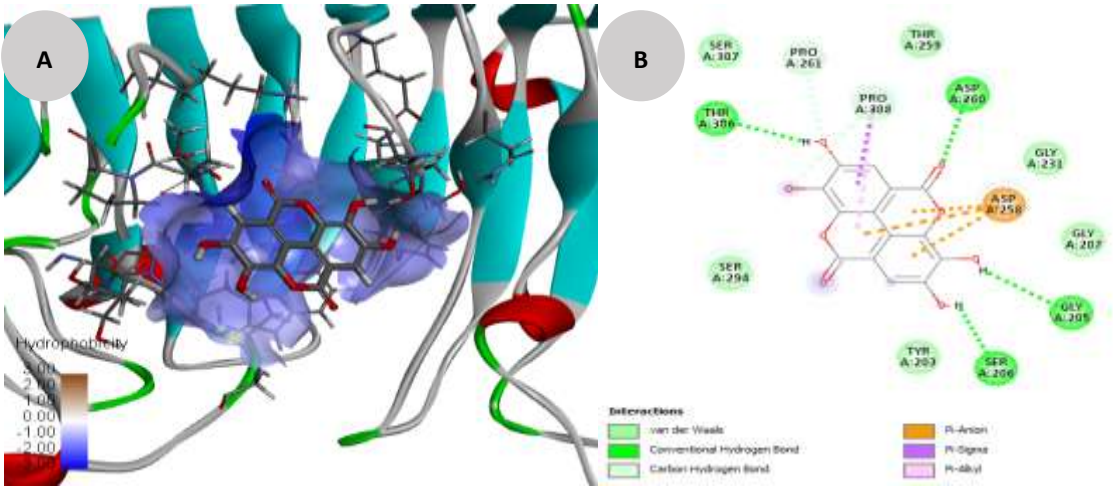


**Figure 2.** (A) Docking Poses and (B) Interaction Maps of Ellagic Acid (Key observation: π-anion catalytic disruption)

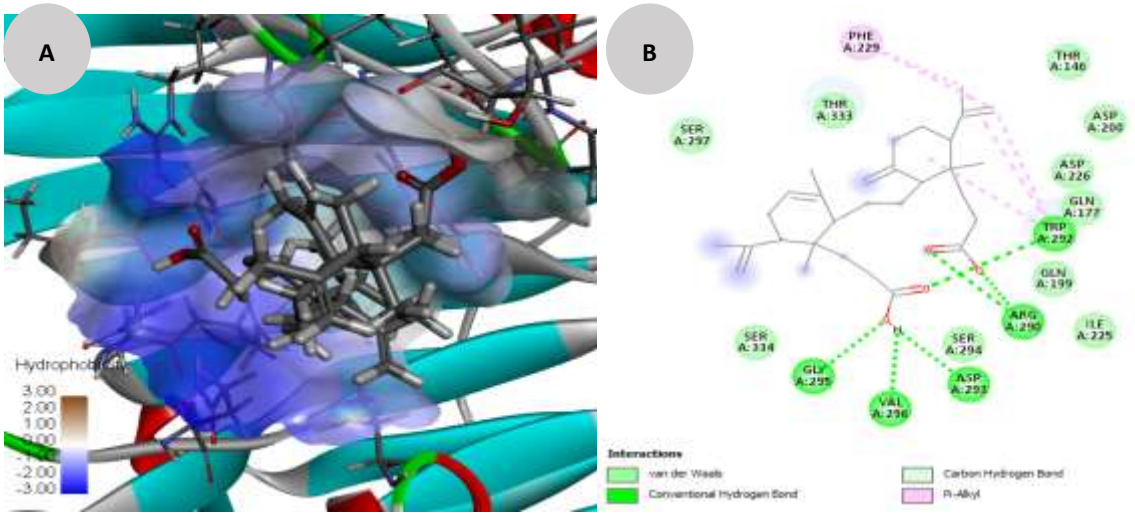


**Figure 3.** (A) Docking Poses and (B) Interaction Maps of Lansic Acid (Key observation: Channel obstruction)

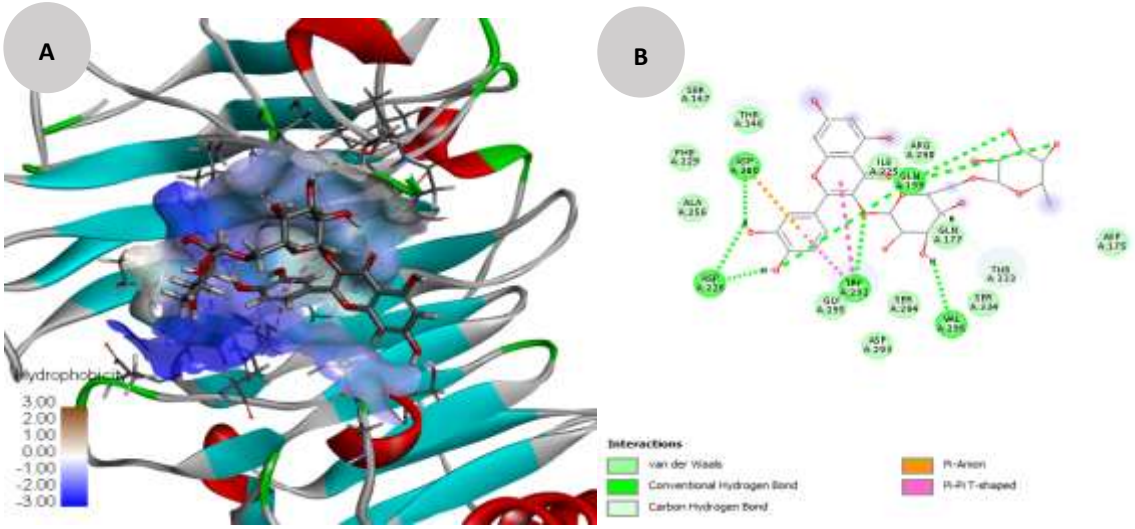


**Figure 4.** (A) Docking Poses and (B) Interaction Maps of Rutin (Key observation: Groove occupation)

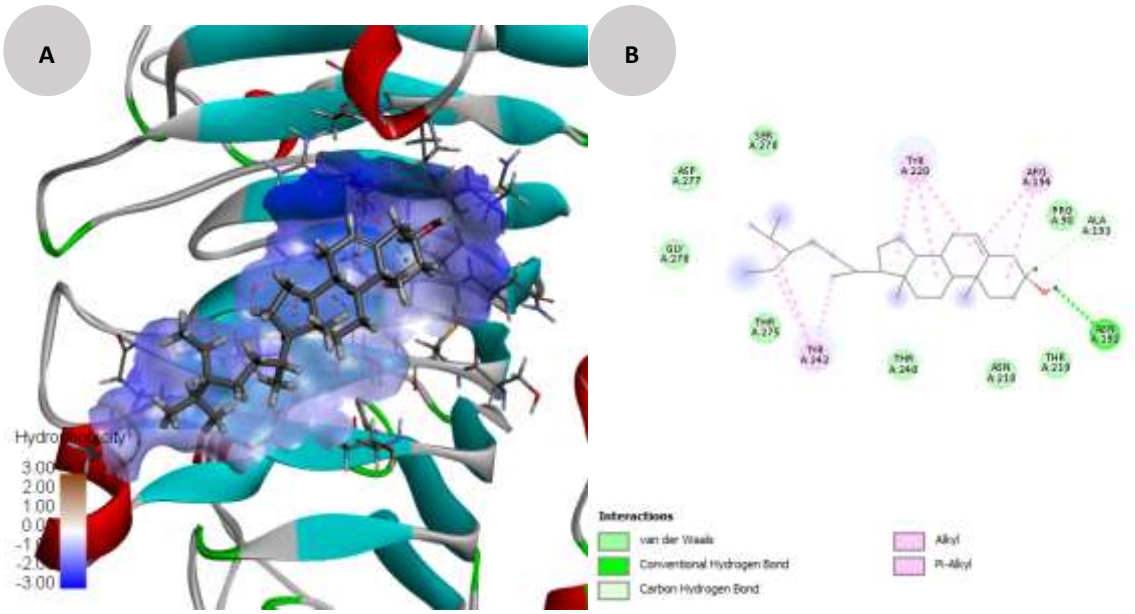


**Figure 5.** (A) Docking Poses and (B) Interaction Maps of β-Sitosterol (Key observation: Surface interference)

The native substrate (Figure 1) adopted a canonical catalytic orientation within the active cleft, forming a directed hydrogen-bond network. Ellagic acid (Figure 2) overlapped the catalytic region and introduced π-anion interference. Lansic acid (Figure 3) blocked the entrance channel through steric occupation. Rutin (Figure 4) extended across the binding groove resembling a polysaccharide chain, whereas β-sitosterol (Figure 5) interacted along the hydrophobic surface interface. The visual agreement between spatial position and predicted mechanism supports the reliability of the interaction-based classification and validates that inhibition is governed primarily by binding topology rather than binding strength.

## DISCUSSION

The present study demonstrates that inhibition of rice weevil pectin methyl esterase (PME) by *L. domesticum* metabolites does not arise from multiple spatially distinct interference mechanisms. Although several ligands exhibited comparable binding affinities, their interaction topology differed markedly, indicating that enzymatic suppression in carbohydrate-active enzymes depends more strongly on substrate positioning than on binding strength alone. PMEs catalyze the demethylesterification of homogalacturonan through a processive mechanism requiring continuous alignment of the polysaccharide chain along an extended binding groove toward catalytic aspartate residues (Kent et al., 2016; Kumar et al., 2023). Consequently, perturbations affecting substrate entry, orientation, catalysis, or release can all reduce digestive efficiency.

Small organic acids such as gallic and citric acid occupied the catalytic cleft similarly to the natural substrate but lacked catalytic orientation. Recent biochemical analyses of plant PMEs have shown that short acidic ligands can slow reaction kinetics by occupying catalytic residues without forming productive enzyme–substrate transition states (Wormit & Usadel, 2018; Coculo & Lionetti, 2022). In the context of insect digestion, such inhibition is expected to decrease nutrient extraction efficiency rather than immediately disable enzyme activity (Chamani et al., 2025).

Ellagic acid displayed a distinct interaction pattern involving direct contacts with catalytic residues through aromatic and electrostatic interactions. Polyphenolic tannins are widely reported to inhibit digestive enzymes by stabilizing non-reactive complexes with catalytic amino acids and altering local electrostatic microenvironments (Martinez-Gonzalez et al., 2017). The observed π–

anion stabilization near catalytic aspartates, therefore, indicates catalytic disruption rather than competitive inhibition (Zhang et al., 2022), suggesting a stronger physiological impact on digestive performance.

Flavonoids such as quercetin and catechin interact primarily with peripheral aromatic residues, forming stacking interactions instead of directly occupying catalytic atoms. Carbohydrate-active enzymes frequently employ aromatic residues as guiding tracks for polymeric substrates; interference at these sites disturbs substrate alignment while leaving the catalytic center structurally intact (Spiwok, 2017; Tian et al., 2023). This mechanism, described here as orientation disruption, likely produces inefficient processing of pectin polymers rather than complete enzyme inactivation.

The triterpenoid lansic acid bound at the entrance channel of the catalytic groove, indicating steric obstruction of substrate access. Similar channel-blocking mechanisms have been described in glycan-processing enzymes where bulky ligands prevent polymer entry into catalytic pockets (Urban et al., 2018; Hrmova & Schwerdt, 2023). Because PME acts on extended polysaccharide chains, blocking the access pathway can suppress enzyme function independently of catalytic chemistry.

Large glycosides such as rutin and lansioside A formed distributed hydrogen-bond networks across the substrate-binding groove, resembling polymer binding but lacking catalytic orientation. Polysaccharide-like inhibitors have been reported to immobilize carbohydrate-processing enzymes by occupying processive binding surfaces, effectively preventing productive catalysis (Schröder et al., 2021). This polymer-mimic mechanism represents a particularly relevant inhibitory strategy because it directly targets the structural requirement for polymer accommodation.

Sterol compounds showed predominantly hydrophobic interfacial association rather than catalytic binding. Amphipathic molecules can alter enzyme–substrate encounter probability by modifying surface interactions at biological interfaces (Azarkina et al., 2023). Such interfacial inhibition may be subtle at the molecular level but biologically significant in heterogeneous digestive environments.

Taken together, six inhibitory modes were identified: competitive mimicry, catalytic disruption, orientation interference, channel blocking, polymer mimicry, and interfacial modulation. Multi-mechanistic interference resembles plant defensive chemistry, where mixtures of metabolites collectively impair herbivore digestion rather than acting as acute toxins (Afroz et al., 2021).

From a biological pest-management perspective, digestive inhibition is particularly relevant for stored-grain pests such as *S. oryzae*. Grain-feeding insects rely on pectin degradation to access nutrients within plant tissues (Roy et al., 2024). Unlike neurotoxic insecticides, digestive inhibitors impose chronic physiological stress, lowering resistance development risk because survival does not depend on a single receptor mutation. Therefore, *L. domesticum* metabolites appear to function as a coordinated digestive defense system that weakens insect physiology by impairing nutrient acquisition rather than causing immediate toxicity.

This study is limited to static molecular docking and post-docking structural interpretation. The protein structure was treated as rigid, which may underestimate conformational flexibility inherent to PME–substrate recognition. Binding affinity values derived from docking scoring functions provide relative rather than quantitative thermodynamic predictions. Additionally, the absence of molecular dynamics simulations prevents evaluation of binding persistence in dynamic aqueous environments, and no in vitro enzymatic validation was performed to confirm inhibitory kinetics.

Future work should integrate molecular dynamics simulations to assess the stability of ligand binding over time and evaluate induced-fit conformational changes. Enzyme kinetics assays using purified insect PME are necessary to quantify inhibition constants and distinguish reversible from

irreversible inhibition. Feeding bioassays in *S. oryzae* could verify physiological effects on digestion and reproduction. Finally, metabolite combinations should be tested, as multi-compound synergy is likely responsible for plant defensive efficacy and may provide an environmentally sustainable strategy for grain protection.

## CONCLUSION

Secondary metabolites of *L. domesticum* inhibit pectin methylesterase of the rice weevil (*S. oryzae*) through multiple mechanisms rather than a single competitive pathway. Six interference modes were identified: catalytic mimicry, catalytic disruption, orientation interference, channel blocking, polymer mimicry, and interfacial modulation. The stability of the protein–ligand complexes indicates that enzymatic suppression results from localized disturbance of substrate processing rather than structural denaturation of the enzyme. These findings suggest that metabolites of *L. domesticum* may function as multi-target digestive inhibitors capable of weakening insect nutrient acquisition. Such a mechanism represents a promising strategy for the development of environmentally friendly botanical pest management agents for stored grains. Future studies integrating molecular dynamics, enzymatic inhibition assays, and insect feeding experiments will be essential to validate the predicted mechanisms and evaluate the practical potential of these metabolites for sustainable grain protection.

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