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*Article*

# Molecular Docking-Based Screening of Phytochemicals from *Dimocarpus longan* Lour. Peel Extract as Potential Larvicidal Agents Against *Aedes aegypti*

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**Abstract.** Dengue fever continues to threaten global health, especially in tropical regions where *Aedes aegypti* mosquitoes proliferate. Ideally, vector populations would be controlled with environmentally safe, effective larvicides. In reality, chemical insecticides like temephos raise concerns about resistance, environmental toxicity, and non-target effects. To address these issues, this study evaluated bioactive compounds from longan peel (*Dimocarpus longan* Lour.) as potential natural larvicides. Thirteen phytochemicals were screened for drug-likeness, toxicity, and aqueous solubility, selecting three compounds with favorable properties. These were docked against 3-HKT, a critical enzyme for larval survival, using AutoDock Vina in PyRx 0.8, with temephos as a reference. Ligand-protein interactions and complex flexibility were analyzed using BIOVIA Discovery Studio and CABS-flex 2.0. Results indicated that epicatechin, 4-O-methylgallic acid, and ethyl gallate exhibited stronger binding affinities than temephos. These findings indicate that phenolic and flavonoid compounds from longan peel can target 3-HKT and may support sustainable mosquito control. These findings highlight the urgency of eco-friendly larvicide development and provide a molecular basis for further in vitro and in vivo validation against Aedes aegypti larvae.



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## 1. Introduction

Dengue fever remains a global public health problem, particularly in tropical and subtropical regions. The World Health Organization reported that, as of April 30, 2024, more than 7.6 million dengue cases had been recorded globally, including 3.4 million confirmed cases, more than 16,000 severe cases, and more than 3,000 deaths [1]. This situation indicates that dengue is not merely a local health issue but also a global public health threat. Indonesia, as a tropical country, has rainfall, humidity, and temperature conditions that support mosquito breeding. In Indonesia, the Ministry of Health reported 88,593 dengue cases with 621 deaths up to the 17$^{th}$ epidemiological week of 2024 [2]. These data confirm that vector control remains an important need in reducing the risk of dengue transmission.

Dengue transmission mainly occurs through the bites of *Aedes aegypti* and *Aedes albopictus mosquitoes. Aedes aegypti* plays a major role because it lives in close association with humans and commonly breeds in water-holding containers around household environments [3]. Larval-stage control is an important strategy because it can interrupt the mosquito life cycle before larvae develop into adult mosquitoes. Therefore, the search for effective, safe, and sustainable larvicidal agents remains an important component of dengue vector control [4].

Synthetic larvicides such as temephos have been widely used to suppress *Aedes aegypti* larval populations. This compound disrupts the larval nervous system mainly by inhibiting acetylcholinesterase (AChE) activity. However, repeated insecticide use may cause new problems, including vector resistance, risks to non-target organisms, and environmental contamination [5]. Recent systematic reviews in Indonesia reported that *Aedes aegypti* populations have shown resistance to organophosphate and pyrethroid insecticides, making environmentally friendly alternatives increasingly necessary [6]. This need also aligns with Sustainable Development Goal 3 (SDG 3): Good Health and Well-being, as dengue vector control directly contributes to reducing the risk of vector-borne diseases [7].

One alternative approach that has been widely developed is the use of plant-based larvicides. Plant bioactive compounds, such as flavonoids, phenolics, alkaloids, saponins, and terpenoids, are known to have potential as mosquito larval control agents [8-9]. These plant-derived compounds may act as larvicidal agents through several mechanisms, including acetylcholinesterase inhibition, disruption of nervous and respiratory functions, membrane and midgut damage, and interference with larval metabolism and development. These effects can lead to feeding inhibition, paralysis, cellular damage, developmental failure, and death of *Aedes aegypti* larvae, suggesting that plant extracts and their derivative compounds may serve as safer biolarvicide candidates than synthetic insecticides when supported by proper efficacy and safety testing [10].

Longan peel (*Dimocarpus longan Lour.*) is an agricultural by-product with potential as a source of bioactive compounds [11]. This fruit peel is often underutilized, although several phytochemical studies have shown that longan pericarp contains phenolic and flavonoid compounds [12]. The compounds selected in this study include Brevifolin, 4-O-methylgallic acid, Proanthocyanidin, Epicatechin, Quercetin, Proanthocyanidins C1, Methyl gallate, Methyl brevifolin carboxylate, and Rutin. These compounds belong mainly to phenolic and flavonoid groups with relevant biological potential, making them suitable candidates for preliminary screening as larvicidal agents against *Aedes aegypti* [13]. The utilization of longan peel also supports Sustainable Development Goal 12 (SDG 12): Responsible Consumption and Production, because it promotes the conversion of agricultural waste into value-added bioactive resources [7].

Biologically, the selection of these compounds has a strong scientific basis. Epicatechin is classified as a flavonoid, while 4-O-methylgallic acid, methyl gallate, and ethyl gallate are phenolic compounds or gallic acid derivatives. Phenolic and flavonoid compounds are often associated with biological activity because they can interact with protein targets through hydrogen bonds, hydrophobic interactions, and electrostatic interactions [14-15]. In the context of larvicidal activity, flavonoids have

been reported to inhibit Noppera-bo protein in *Aedes aegypti*, an enzyme involved in the biosynthesis of ecdysone, a hormone essential for larval development [16].

The in silico approach is a relevant preliminary method for selecting potential antilarvicidal compounds. This method allows researchers to predict interactions between bioactive compounds and biological targets before laboratory testing. One commonly used technique is molecular docking, a computational simulation used to evaluate binding patterns, binding energy, and ligand-target affinity [17-18]. In larvicidal research, molecular docking has been used to evaluate plant-derived compounds against important *Aedes aegypti* targets, including AChE, Noppera-bo, and 3-hydroxykynurenine transaminase (3-HKT) [16].

Among these molecular targets, 3-HKT was selected as the main target in this study because of its essential role in mosquito larval survival. This enzyme is involved in the tryptophan catabolism pathway by converting toxic 3-HKT into the more stable xanthurenic acid (XA) [19]. In *Aedes aegypti*, excessive accumulation of 3-HK may induce oxidative stress, neuronal damage, paralysis, and larval death. Therefore, inhibition of 3-HKT can disrupt an important detoxification pathway required for larval development and survival [20]. In addition, 3-HKT is considered a relevant mosquito target because it has low sequence similarity with human kynurenine aminotransferases (KAT-I) and II (KAT-II) [21]. These biological characteristics provide a strong rationale for selecting 3-HKT as the primary protein target in this study.

Previous studies have shown that the combination of in silico approaches and biological assays can support the discovery of new larvicidal candidates for example, screened plant secondary metabolites through molecular docking against 3-HKT in *Aedes aegypti* [22]. Their screening identified a chalcone compound with strong binding energy, which was later confirmed through in vitro testing to have high larvicidal activity. This finding indicates that molecular docking can serve as a rational preliminary step to narrow down candidate compounds before further experimental validation.

Based on the above explanation, longan peel has potential as a source of natural antilarvicidal candidates because it contains bioactive compounds such as 4-O-methylgallic acid, methyl gallate, ethyl gallate, and epicatechin. However, studies on the molecular interactions between these compounds and target proteins in *Aedes aegypti* larvae remain limited. Therefore, this study aims to screen antilarvicidal compounds from longan peel extract (*Dimocarpus longan Lour*.) through an in silico approach using molecular docking. The findings are expected to identify candidate compounds with the best binding affinity toward selected target proteins and provide an initial basis for developing more effective and environmentally friendly natural biolarvicides. This study also contributes to SDG 3 through dengue vector control and SDG 12 through the valorization of agricultural by-products.

## 2. Experimental Section

### 2.1. Materials

This study utilized hardware in the form of a laptop with the following specifications: Intel® Core™ i3-10110U CPU @ 2.10 GHz, 4 CPUs, 2.6 GHz, and 4.00 GB RAM. The software used included Windows operating system, PyRx 0.8, PyMOL 1.7.4.5, BIOVIA Discovery Studio 2024 Client 24.1.0, PubChem, RCSB, Lipinski's Rule of Five, CABS-flex 2.0, and ProTox 3.0.

The selected test ligands were determined based on primary phytochemical studies reporting the presence of phenolic and flavonoid compounds in longan peel or pericarp (*Dimocarpus longan Lour.).* The PubChem CID, chemical class, reported plant source, and supporting primary references of each ligand are summarized in Table 1.

**Table 1.** Test Ligands

| No. | Bioactive compound name | CID | Chemical class | Role | Reported source | Recent supporting reference |
|---|---|---|---|---|---|---|
| 1 | Temephos | 5,392 | Organophosphate | Control ligand | Not applicable | Control ligand, not derived from longan peel |
| 2 | Gallic acid | 370 | Phenolic acid | Test ligand | Longan byproduct/peel | Tan et al. (2023) [23]; Chollakup et al. (2021) [24] |
| 3 | Ellagic acid | 5,281,855 | Phenolic compound | Test ligand | Longan peel/pericarp | Chollakup et al. (2021) [24]; Hu et al. (2025) [25] |
| 4 | 4-O-methylgallic acid | 78,016 | Phenolic acid derivative | Test ligand | Longan pericarp | Bai et al. (2019) [26] |
| 5 | Protocatechuic acid | 72 | Phenolic acid | Test ligand | Longan pericarp | Bai et al. (2019) [26] |
| 6 | Isoscopoletin | 69,894 | Coumarin derivative | Test ligand | Longan pericarp | Bai et al. (2019) [26] |
| 7 | Corilagin | 73,568 | Hydrolysable tannin | Test ligand | Longan pericarp/byproduct | Tang et al. (2019) [27] |
| 8 | Methyl gallate | 7,428 | Gallic acid derivative | Test ligand | Longan pericarp | Paul et al. (2021) [28] |
| 9 | Ethyl gallate | 13,250 | Gallic acid derivative | Test ligand | Longan pericarp/byproduct | Tang et al. (2019) [27] |
| 10 | Epicatechin | 72,276 | Flavonoid | Test ligand | Longan peel/pericarp | Tan et al. (2023) [23]; Chollakup et al. (2021) [24] |
| 11 | Quercetin | 5,280,343 | Flavonoid | Test ligand | Longan pericarp/peel | Bai et al. (2019) [26] |
| 12 | Proanthocyanidins C1 | 169,853 | Condensed tannin/flavonoid | Test ligand | Longan pericarp/byproduct | Tan et al. (2023) [23]; Paul et al. (2021) [28] |
| 13 | Rutin | 5,280,805 | Flavonoid glycoside | Test ligand | Longan pericarp | Bai et al. (2019) [26] |
| 14 | Methyl brevifolin carboxylate | 5,319,518 | Phenolic derivative | Test ligand | Longan pericarp | Paul et al. (2021) [28] |

## 2.2. Methods

This infographic illustrates the stepwise *in silico* workflow for screening bioactive compounds from longan peel (*Dimocarpus longan* Lour.) as potential larvicidal agents against *Aedes aegypti*. It presents five stages: initial research, ligand and protein preparation, implementation of molecular docking, molecular interaction and simulation, and final evaluation. Each stage details key steps such as literature review, selection and preparation of ligands, docking with AutoDock Vina, interaction analysis using PyMOL and BIOVIA Discovery Studio, protein flexibility assessment with CABS-flex 2.0, and final selection based on binding affinity, interactions, residue-level flexibility, toxicity, and solubility. The flowchart uses a vertical layout, clear arrows, scientific icons, and a blue-green color palette, providing a professional, journal-ready visualization of the computational methodology.

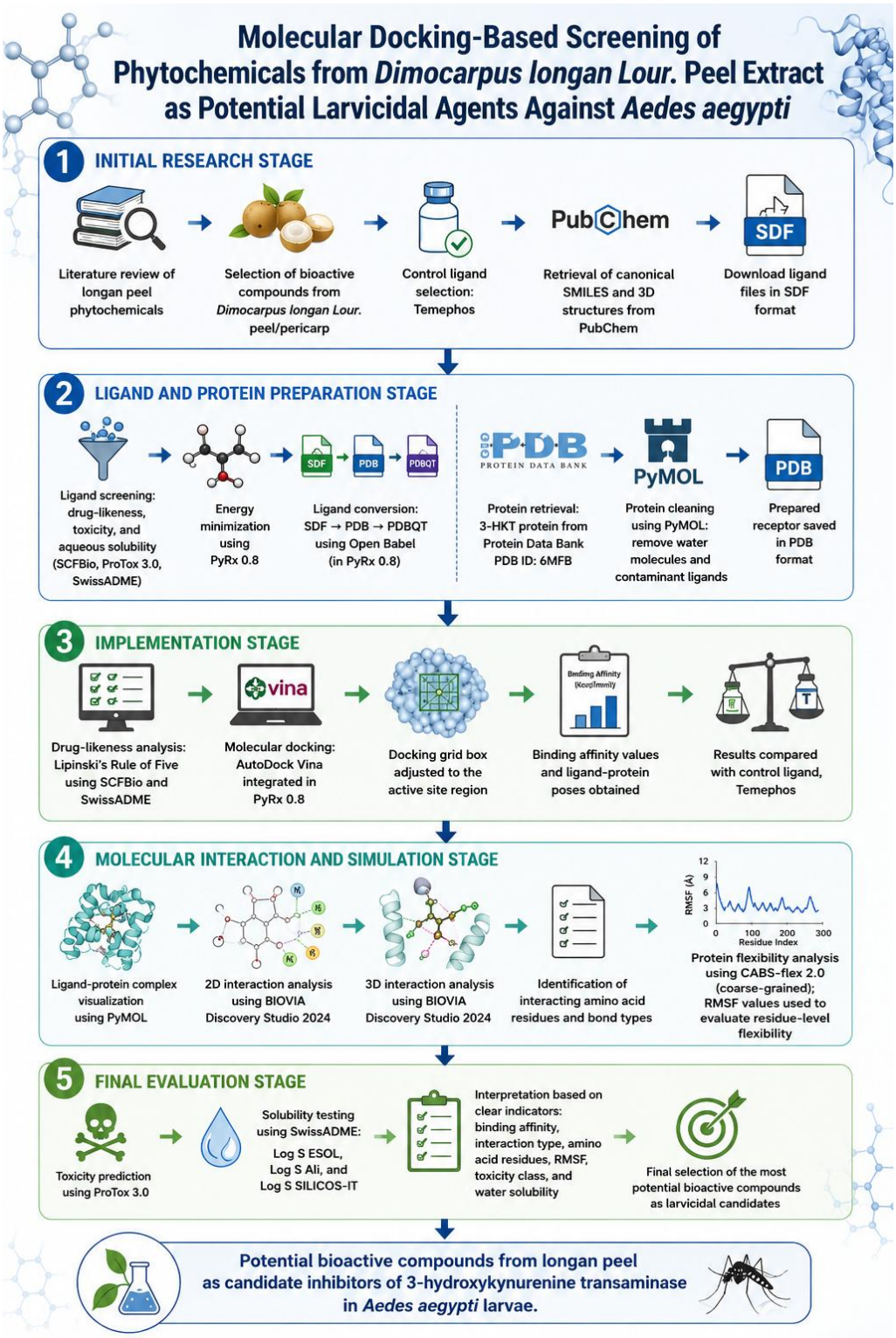


**Figure 1.** Molecular Docking-Based Screening of Phytochemicals from *Dimocarpus longan* Lour. Peel Extract as Potential Larvicidal Agents Against *Aedes aegypti*

### 2.2.1. Preparation of Test Ligands

The in silico approach is widely used as an initial screening method to evaluate ligand-protein interactions, binding affinity, drug-likeness, and ADMET properties before experimental validation [29]. Based on this rationale, the test ligands in this study were selected from previous phytochemical studies that reported the presence of bioactive compounds in longan peel or pericarp (*Dimocarpus longan Lour.*), particularly phenolic and flavonoid compounds [23]. A total of 13 plant-derived bioactive compounds and one control ligand were initially included in this study. Temephos was used as the control insecticide because it is commonly applied to control *Aedes aegypti* larvae and served as a reference compound for comparing the binding affinity of the selected ligands toward the target enzyme.

The canonical SMILES and three-dimensional structures of the plant-derived compounds and temephos were obtained from the PubChem database and downloaded in SDF format. Prior to molecular docking, the compounds were screened for drug-likeness, predicted toxicity, and aqueous solubility. Only compounds that met all screening criteria were selected for docking analysis. The three-dimensional structures of the selected ligands were energy-minimized and converted into PDB format using PyRx 0.8. The minimized ligand structures were then converted into AutoDock ligand format, namely PDBQT, using Open Babel integrated in PyRx 0.8. The prepared PDBQT ligand files were used as input for subsequent molecular docking analysis.

### 2.2.2. Protein Preparation

Following ligand preparation, the next step was the preparation of the target protein as the receptor. The 3D structure of 3-hydroxykynurenine transaminase (3-HKT) was obtained from the Protein Data Bank (https://www.rcsb.org) with PDB ID: 6MFB and downloaded in PDB format. Protein preparation was performed using PyMOL 1.7.4.5 to remove water molecules and ligands before further analysis. First, PyMOL + Tcl-Tk GUI + Console was opened, the downloaded protein was loaded, water molecules were removed via the "Remove Water" function, and any contaminant ligands were identified using the "Ligand Sites" preset and removed. The cleaned protein was then saved using "Save Molecule."

### 2.2.3. Drug-Likeness Analysis

The bioactive compounds were evaluated for drug-likeness using Lipinski's Rule of Five on the SCFBio server (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The criteria included: molecular weight <500 Da, LogP <5 to ensure moderate lipophilicity, hydrogen bond donors <5, hydrogen bond acceptors <10, and molar refractivity between 40–130. This assessment helped characterize ligands based on their similarity to known drug molecules. Additional analysis could be performed using SwissADME (http://www.swissadme.ch/).

### 2.2.4. Molecular Docking

Molecular docking is commonly used in *in silico* studies to predict ligand-protein binding affinity, binding poses, and molecular interactions, including hydrogen bonds and hydrophobic interactions. This approach also complements drug-likeness and ADMET-based screening in the early evaluation of natural compounds [30]. In this study, molecular docking was performed using AutoDock Vina integrated in PyRx 0.8 to evaluate the binding affinity between the selected ligands and 3-hydroxykynurenine transaminase (3-HKT). The prepared ligand files in PDBQT format and the prepared target protein were imported into PyRx 0.8. The docking grid box was adjusted to cover the active site region of the target protein. Docking analysis was conducted to obtain binding affinity values and predicted ligand-protein binding poses. The docking results were compared with temephos as the control ligand. The ligand-protein interactions were further visualized and analyzed using BIOVIA Discovery Studio.

### 2.2.5. Molecular Interaction Analysis

Docked ligands were combined with the prepared protein in PyMOL to verify proper binding. Ligand-protein complexes were visualized and saved. Detailed interactions were analyzed using BIOVIA Discovery Studio 2024 Client by generating publication-quality images, labeling interacting amino acids, and creating 2D diagrams to observe bond types. Results were saved as Discovery Studio files and 2D images.

### 2.2.6. Molecular Dynamics Analysis

The flexibility of the ligand-protein complex was evaluated using CABS-flex 2.0 (http://biocomp.chem.uw.edu.pl/CABSflex2/submit) through a coarse-grained protein flexibility simulation. The analysis was performed based on root mean square fluctuation (RMSF) values to observe residue-level flexibility in the predicted ligand-protein complex. This analysis was not intended to represent a conventional all-atom molecular dynamics simulation such as those performed using GROMACS or AMBER.

### 2.2.7. Toxicity Prediction

Bioactive compounds and the control were evaluated for toxicity using ProTox 3.0 (https://tox.charite.de/protox3/), which predicts small-molecule toxicity. Compounds were entered by name or canonical SMILES, and all available predictive models were selected. Results were obtained after processing.

### 2.2.8. Solubility Testing

The aqueous solubility of bioactive compounds and the control was determined using three methods: Log S (ESOL), Log S (Ali), and Log S (SILICOS-IT) via SwissADME (http://www.swissadme.ch/). The canonical SMILES of the selected compounds were entered, the "Run" function was executed, and solubility values in water were recorded.

## 3. Results and Discussion

### 3.1. Drug-Likeness, Toxicity, and Solubility Results

Prior to molecular docking, 13 phytochemicals from longan peel (*Dimocarpus longan Lour*.) and one control ligand were screened for drug-likeness, toxicity, and aqueous solubility. Four phytochemicals, namely 4-O-methylgallic acid, methyl gallate, ethyl gallate, and epicatechin, met the screening criteria for drug-likeness, low toxicity, and water solubility; therefore, they were selected for molecular docking, while temephos was retained as the control ligand.

In general, these compounds interacted with the 3-HKT enzyme through various molecular interactions, including conventional hydrogen bonds, carbon-hydrogen bonds, π-cation, π-anion, π-π stacked, π-π T-shaped, π-alkyl, and π-sulfur interactions and unfavorable interactions. Toxicity classification was determined based on the predicted $LD_{50}$ values according to the Globally Harmonized System (GHS). The $LD_{50}$ value indicates the estimated dose required to cause death in 50% of test subjects, expressed in mg/kg body weight. Lower $LD_{50}$ values indicate greater toxicity, whereas higher values indicate lower toxicity. Toxicity was classified into six GHS classes. Class 1 indicates extreme toxicity with LD50 ≤ 5 mg/kg. Class 6 indicates compounds with low toxicity with $LD_{50}$ > 5,000 mg/kg. Safer compounds were prioritized for docking.

The analysis confirmed that four phytochemicals and the control ligand complied with Lipinski's Rule of Five, indicating their potential drug-likeness. These rules help evaluate compounds based on molecular characteristics that predict effective distribution in the body and assess their suitability as drug candidates. The criteria include molecular weight <500 Da, LogP <5, H-bond donors <5, H-bond acceptors <10, and molar refractivity between 40–130. Molecules exceeding 500 Da may have reduced membrane permeability, limiting absorption. Compounds with LogP <5 are more likely to

be absorbed, while LogP >5 indicates higher lipophilicity and potential toxicity. H-bond donors and acceptors correlate with the energy required for absorption, and molar refractivity indicates suitability for effective interaction with biological targets and membrane penetration [31].

**Table 2.** Drug-Likeness, Toxicity, and Solubility of Compounds

| No | Compound | Drug-Likeness | $LD_{50}$ (mg/kg) | Toxicity Class | Solubility |
|---|---|---|---|---|---|
| 1 | Epicatechin | Drug-like | 10,000 | 6 | Soluble |
| 2 | 4-O-methylgallic acid | Drug-like | 5,000 | 5 | Soluble |
| 3 | Ethyl gallate | Drug-like | 5,810 | 6 | Soluble |
| 4 | Methyl gallate | Drug-like | 1,700 | 4 | Soluble |
| 5 | Temephos (control) | Drug-like | 1,190 | 4 | Insoluble |

Toxicity predictions were conducted using ProTox 3.0, providing $LD_{50}$ values and toxicity classification. Higher $LD_{50}$ indicates lower toxicity. Among the compounds, ethyl gallate ($LD_{50}$ = 5,810 mg/kg) and epicatechin ($LD_{50}$ = 10,000 mg/kg) belonged to toxicity class 6, while methyl gallate ($LD_{50}$ = 1,700 mg/kg) and the control temephos ($LD_{50}$ = 1,190 mg/kg) were class 4. Water solubility (Log S) influences the absorption and distribution of biologically active compounds. Solubility was assessed using three methods: Log S (ESOL), Log S (Ali), and Log S (SILICOS-IT) via SwissADME. All four selected phytochemicals were hydrophilic, whereas temephos was hydrophobic, while temephos was hydrophobic. Hydrophilic compounds, except temephos, were able to cross cell membranes and potentially inhibit 3-HKT via transporters or structural modifications, exhibiting strong affinity [32].

### 3.2. Molecular Docking Results

Molecular docking was performed to evaluate the binding potential of the compounds with the 3-HKT receptor. Temephos, the control ligand, exhibited a binding affinity of -5.9 kcal/mol, while the four selected longan peel compounds showed binding affinities ranging from -5.8 to -9.3 kcal/mol. Binding affinity values indicate the stability of ligand-receptor interactions; more negative values suggest stronger and spontaneous binding. The docking ranking from strongest to weakest binding was epicatechin (-9.3 kcal/mol), 4-O-methylgallic acid (-6.8 kcal/mol), ethyl gallate (-6.3 kcal/mol), temephos (-5.9 kcal/mol), and methyl gallate (-5.8 kcal/mol). Compounds with more negative binding affinity than the control demonstrated specific interaction with the 3-HKT binding site.

**Table 3.** Molecular Docking Results

| No | Ligand | Protein | Binding Affinity (kcal/mol) |
|---|---|---|---|
| 1 | Epicatechin | 3-HKT | -9.3 |
| 2 | 4-O-methylgallic acid | 3-HKT | -6.8 |
| 3 | Ethyl gallate | 3-HKT | -6.3 |
| 4 | Methyl gallate | 3-HKT | -5.8 |
| 5 | Temephos (control) | 3-HKT | -5.9 |

Epicatechin showed the strongest binding affinity against 3-HKT, with a binding energy of -9.3 kcal/mol. This stronger affinity can be explained by its chemical structure. Epicatechin is a flavanol that contains several hydroxyl groups on its aromatic rings. These hydroxyl groups provide hydrogen-bond donor and acceptor sites, allowing epicatechin to form stronger and more stable interactions

with amino acid residues in the 3-HKT binding pocket. In addition, its aromatic ring system may support π-based interactions, which further stabilize the ligand-protein complex. Compared with smaller phenolic acids such as vanillic acid, methyl gallate, 4-O-methylgallic acid, and ethyl gallate, epicatechin has a larger polyphenolic framework and more interaction sites. This structural feature may explain why epicatechin produced the lowest binding energy among the tested compounds [33].

The result is also consistent with phytochemical studies on longan pericarp. Longan pericarp is rich in phenolic compounds and identified several pericarp-derived phenolics, including quercetin, ellagic acid, corilagin, and proanthocyanidins C1. The same study also stated that earlier studies had detected 4-O-methylgallic acid and epicatechin in longan pericarp. Gallic acid, ethyl gallate, corilagin, and ellagic acid in longan pericarp and seed extracts using HPLC. Longan pericarp polyphenol extract contains gallic acid, proanthocyanidin B2, epicatechin, proanthocyanidin A2, and rutin. These findings support the selection of epicatechin and other phenolic compounds as ligands derived from longan peel or pericarp.

Compared with previous experimental studies, direct in vitro or in vivo evidence on the larvicidal activity of longan peel extract against *Aedes aegypti* remains limited. Therefore, the present in silico result should be interpreted as an initial molecular prediction rather than definitive biological proof. However, the result agrees with the broader evidence that plant phenolics and flavonoids can contribute to larval toxicity through disruption of essential enzymes, oxidative balance, membrane integrity, and metabolic processes. The strong binding of epicatechin to 3-HKT suggests that this compound may interfere with the kynurenine pathway by inhibiting the conversion of toxic 3-hydroxykynurenine into xanthurenic acid. Such inhibition may promote toxic metabolite accumulation and disturb larval survival [34]. Thus, epicatechin may be one of the key longan peel constituents responsible for potential larvicidal activity, although further in vitro larval mortality assays and in vivo toxicity studies are required to confirm this mechanism.

### 3.3. Molecular Interaction and Dynamics Analysis

Figure 1 shows the Root Mean Square Fluctuation (RMSF) plot for the complex of 3-HKT protein with the control ligand, temephos. The X-axis represents the residue index of amino acids, while the Y-axis indicates RMSF values in Ångström, reflecting the flexibility of each residue during molecular dynamics simulation. Residues with RMSF values below 3 Å indicate stable interactions, showing that temephos consistently binds to the active site without significant structural changes. Peaks in the plot correspond to more flexible residues, while troughs represent more rigid regions, confirming overall stability of the ligand-protein complex.

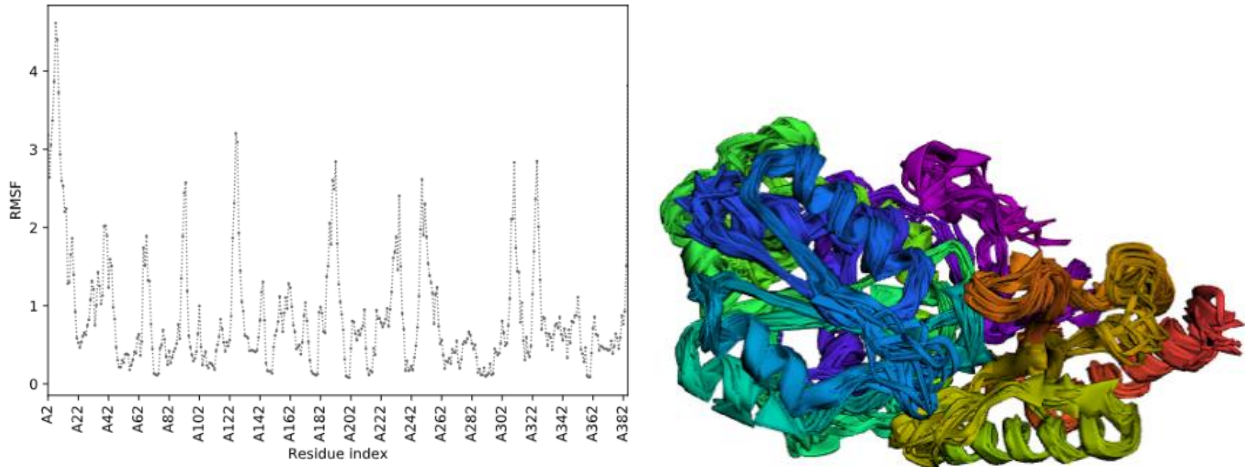


**Figure 1.** Molecular Interaction of Temephos (Control) and 3-HKT Protein

Figure 2 presents the Root Mean Square Fluctuation (RMSF) plot for the 3-HKT protein in complex with epicatechin. The X-axis represents the residue index of amino acids, while the Y-axis shows RMSF values in Ångström, indicating the flexibility of each residue during molecular dynamics simulation. RMSF values below 3 Å demonstrate stable binding interactions, suggesting that epicatechin maintains consistent contact with the active site without disrupting protein structure. Peaks in the graph reflect more flexible residues, whereas troughs indicate rigid regions, confirming the overall stability of the epicatechin–3-HKT complex.

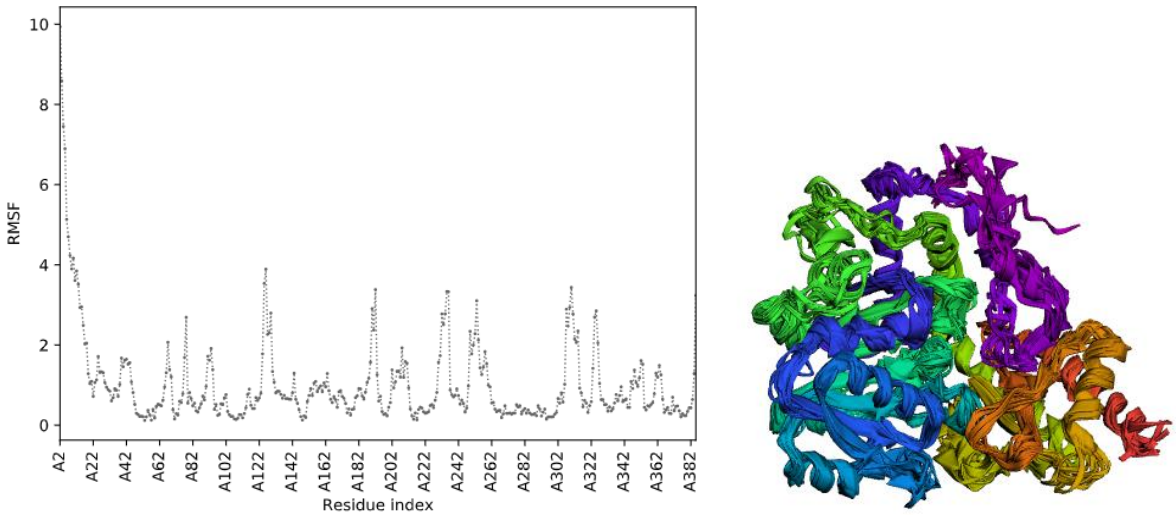


**Figure 2.** Molecular Interaction of Epicatechin and 3-HKT Protein

Figure 3 presents the Root Mean Square Fluctuation (RMSF) plot for the 3-HKT protein in complex with 4-O-methylgallic acid. The X-axis represents the residue index of amino acids, while the Y-axis shows RMSF values in Ångström, indicating the flexibility of each residue during molecular dynamics simulation. RMSF values below 3 Å for most residues suggest stable binding of 4-O-methylgallic acid to the protein's active site without causing significant structural changes. Peaks indicate more flexible residues, whereas troughs correspond to rigid regions, confirming the overall stability of the 4-O-methylgallic acid–3-HKT complex.

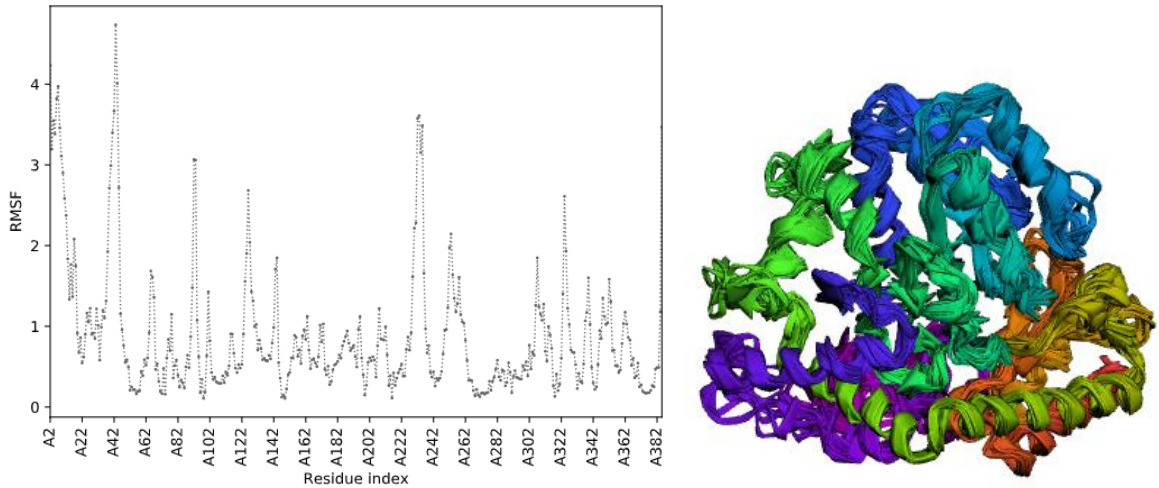


**Figure 3.** Molecular Interaction of 4-O-Methylgallic Acid and 3-HKT Protein

Figure 4 displays the Root Mean Square Fluctuation (RMSF) plot for the 3-HKT protein in complex with ethyl gallate. The X-axis indicates the residue index of amino acids, while the Y-axis shows RMSF values in Ångström, representing the flexibility of each residue during molecular dynamics simulation. Most RMSF values are below 3 Å, suggesting that ethyl gallate binds stably to the active site of 3-HKT without causing significant structural changes. Peaks reflect more flexible residues, whereas troughs represent more rigid regions, confirming the overall stability of the ethyl gallate–3-HKT complex.

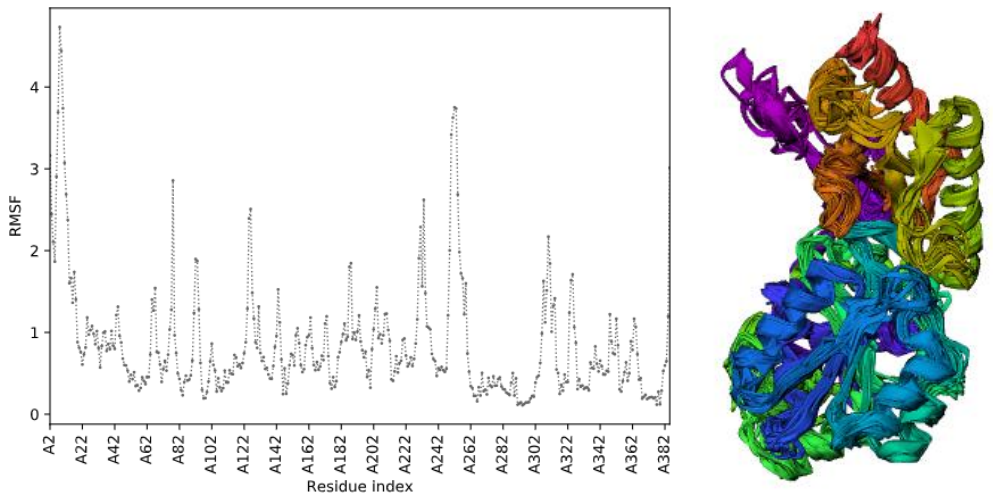


**Figure 4.** Molecular Interaction of Ethyl Gallate and 3-HKT Protein

The ligands selected for further analysis included three phytochemicals: epicatechin, 4-O-methylgallic acid, and ethyl gallate. Which exhibited more negative binding affinities than the control ligand, temephos (-5.9 kcal/mol). These three phytochemicals and the control ligand were then subjected to molecular dynamics analysis to molecular dynamics simulations, and the results showed RMSF values below 3 Å, indicating stable interactions. The molecular interactions between the test compounds and the 3-HKT protein were visualized using both two-dimensional (2D) and three-dimensional (3D) diagrams. The 3D visualization displayed the receptor surface in green and pink, with pink indicating hydrogen bond donors and green representing hydrogen bond acceptors. The 2D visualization illustrated all molecular interactions in a single diagram, including symbols and color codes to denote interaction types.

The most stable bond distance was observed at residue THR259 (2.35 Å) for 4-O-methylgallic acid. In contrast, ethyl gallate exhibited the longest bond distance among the ligands, 5.52 Å at residue PHE347. Shorter distances indicate stronger and more stable interactions, while longer distances correspond to weaker and less stable bonds.

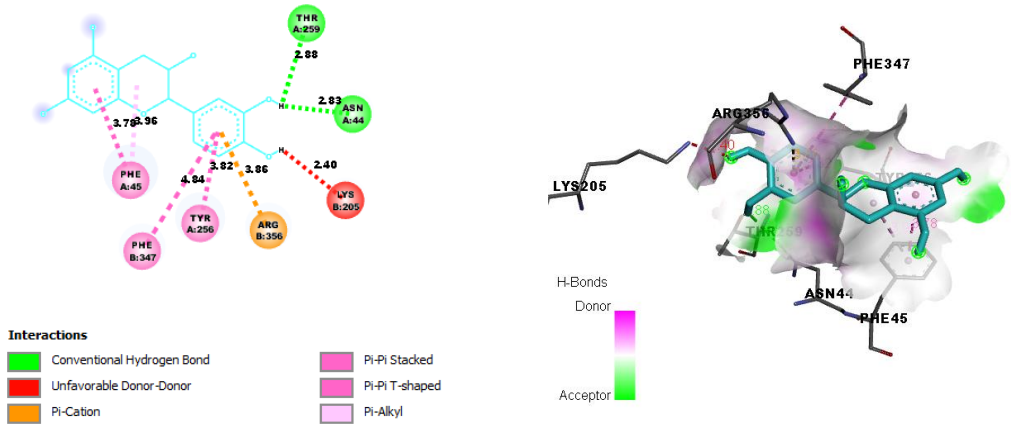


**Figure 6.** 2D and 3D Visualization of Epicatechin Compound with 3-HKT protein

Figure 6 illustrates the molecular interaction analysis of the epicatechin compound with the 3-HKT protein, showing interactions with amino acid residues at the active site, namely ASN44, THR259, PHE45, TYR256, PHE347, ARG356, and LYS205.

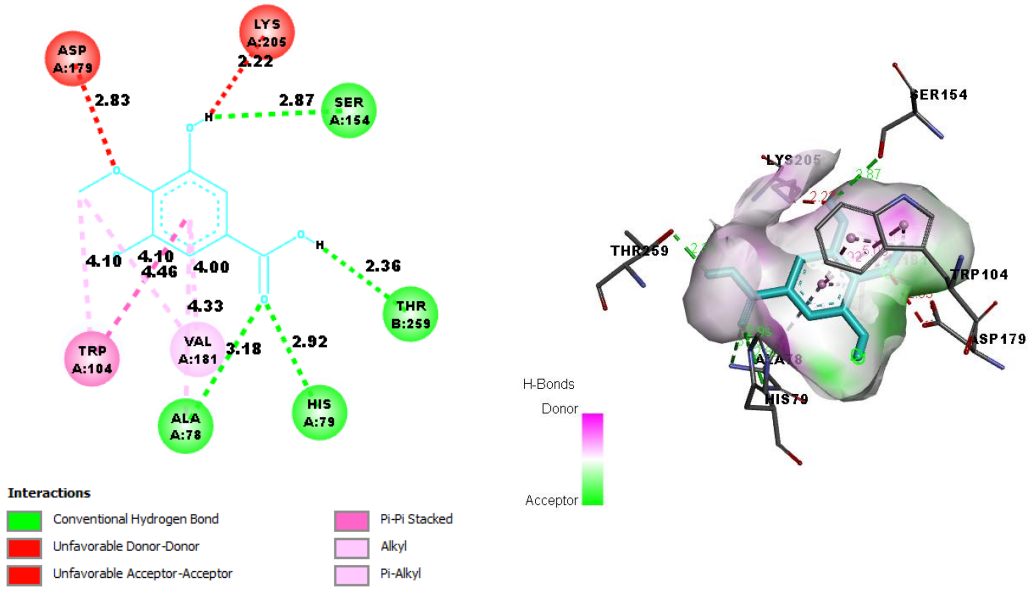


**Figure 7.** 2D and 3D Visualization of 4-O-methylgallic acid Compound with 3-HKT Protein

Figure 7 shows the molecular interaction analysis of the 4-O-methylgallic acid compound with the 3-HKT protein, revealing interactions with amino acid residues at the active site, namely ALA78, HIS79, THR259, SER154, TRP104, VAL181, ASP179, and LYS205.

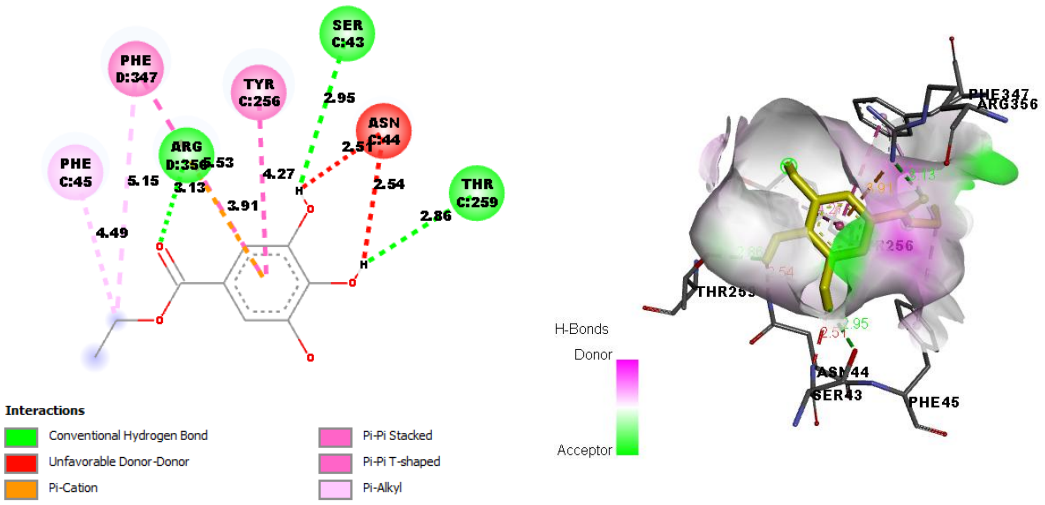


**Figure 8.** 2D and 3D Visualization of Ethyl Gallate Compound with 3-HKT Protein

Figure 8 shows the molecular interaction analysis of the ethyl gallate compound with the 3-HKT protein, revealing interactions with amino acid residues at the active site, namely ARG356, SER43, THR259, TYR256, PHE347, PHE45, and ASN44.

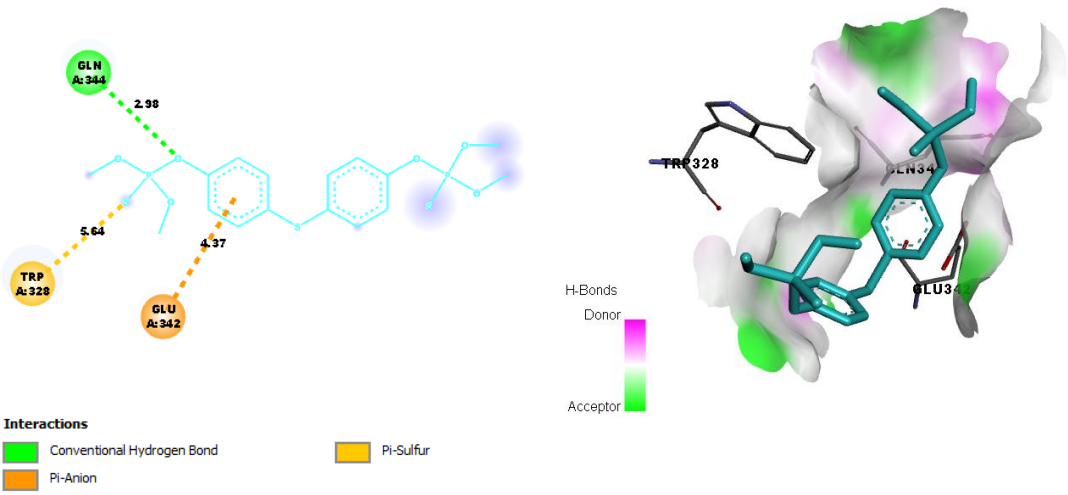


**Figure 9.** 2D and 3D Visualization of Temephos with 3-HKT Protein

Figure 9 shows the molecular interaction analysis of the control ligand temephos with the 3-HKT protein, revealing interactions with amino acid residues at the active site, namely GLN344, GLU342, and TRP328. The interaction analysis of each ligand revealed the formation of hydrogen bonds, hydrophobic interactions, and electrostatic interactions. The order of bond strength from strongest to weakest is hydrogen bonds, hydrophobic interactions, and electrostatic interactions. Hydrogen bonds play a crucial role in maintaining protein stability and folding. The protein's secondary structures, such as α-helices and β-sheets, contain hydrogen bonds that are essential for proper protein folding [35], [36]. Even when the distance between the compound and the receptor is relatively large, hydrogen bonds can still form. A higher number of hydrogen bonds increases the

compound's binding affinity to the receptor, making it a strong candidate as an effective inhibitor [37], [38].

The test compound epicatechin and the two other ligands exhibited the same three types of interactions: hydrogen bonds, hydrophobic interactions, and electrostatic interactions. The difference lies in the number of amino acid residues involved in interactions with the 3-HKT protein. Epicatechin was the strongest ligand compared to the control temephos and the other ligands, interacting with a total of six amino acid residues: ASN44, THR259, PHE45, TYR256, PHE347, and ARG356.

Hydrophobic interactions between 3-HKT protein and the ligands (Table 4) showed that almost all ligands and the control exhibited similar types of hydrophobic interactions, including π-π T-shaped, π-alkyl, π-anion, and π-π stacked interactions. Although relatively weak, hydrophobic interactions play an important role in ligand-receptor binding [37]. These interactions occur when two hydrophobic groups interact and are crucial in determining the stability of the ligand-receptor complex. Hydrophobic residues tend to avoid aqueous environments and cluster within the protein's globular structure. Residues involved in hydrophobic interactions are typically nonpolar amino acids [38], [39].

Both hydrophobic and hydrogen bond interactions play a key role in maintaining the stability of active compound binding to the target protein. A high number of hydrogen bonds supported by hydrophobic interactions can result in a strong ligand-receptor interaction [40]. Electrostatic interactions between 3-HKT protein and the ligands (including temephos as the control) involved π-cation and π-anion types. These interactions arise from polarity differences. Although weak and non-covalent, electrostatic interactions contribute significantly to the formation of a stable protein conformation due to their abundance [39].

**Table 4.** Analysis of 3-HKT Protein Docking Results with Test Compounds

| No | Test Compound | Hydrogen Bonds | Hydrophobic Interactions | Electrostatic Interactions | RMSF |
|---|---|---|---|---|---|
| 1 | Epicatechin | ASN44 and THR259 | PHE45, TYR256, PHE347 | ARG356 | Stable |
| 2 | 4-O-methylgallic acid | ALA78, HIS79, THR259, SER154 | TRP104, VAL181, ALA78 | - | Stable |
| 3 | Ethyl gallate | ARG356, SER43, THR259 | TYR256, PHE347, PHE45 | ARG356 | Stable |
| 4 | Temephos (control) | GLN344 | - | GLU342 | Stable |

Epicatechin is a flavanol compound belonging to the flavonoid group. Flavonoids are plant-derived polyphenolic compounds that have been widely associated with biological activities, including antioxidant, anti-inflammatory, and anticancer-related effects [41], [42]. In this study, surface visualization from molecular docking showed that epicatechin had the strongest affinity toward the 3-HKT protein compared with the control ligand, temephos. Epicatechin formed hydrogen bonds, hydrophobic interactions, and electrostatic interactions with six amino acid residues at the active site of 3-HKT. These interactions contributed to its high binding affinity and indicated its potential as a 3-HKT inhibitor.

4-O-methylgallic acid is a major metabolite derived from hydrolyzed gallic acid tannins [43]. This compound forms only two types of bonds, hydrogen and hydrophobic, resulting in lower docking energy compared to epicatechin. Ethyl gallate, the ethyl ester of gallic acid, is known as an antioxidant

[44]. Molecular docking demonstrated that it has potential to inhibit the 3-HKT enzyme, showing significant binding energy and residue interactions similar to the other ligands.

The stability of molecular interactions was assessed using the Root Mean Square Fluctuation (RMSF) score calculated for the ligand-protein complexes. RMSF values below 3 Å were considered stable. The molecular interaction analysis showed that the test compounds epicatechin, 4-O-methylgallic acid, and ethyl gallate, as well as the control compound temephos, all had RMSF values below 3 Å. This indicates that all compounds have the potential to act as effective inhibitors, consistently binding to the 3-HKT protein without causing structural alterations. RMSF values not only reflect structural conformation fluctuations during protein activity but also measure the extent to which atomic positions deviate from their initial locations, thereby reflecting the dynamic nature of the interaction between the protein and the test compound. In other words, RMSF indicates the degree of interaction dynamics. A higher number of residues or groups with elevated RMSF values suggests greater flexibility, indicating a higher likelihood of interaction with the ligand. Conversely, lower RMSF fluctuations correspond to reduced flexibility, thereby limiting potential interactions [45].

## 4. Conclusion

Based on the in silico analysis, three bioactive compounds from longan peel, namely epicatechin, 4-O-methylgallic acid, and ethyl gallate demonstrated strong inhibitory potential against 3-hydroxykynurenine transaminase (3-HKT). These compounds formed stable hydrogen bonds, hydrophobic interactions, and electrostatic interactions at the enzyme's active site, with stronger binding affinities than temephos and RMSF values below 3 Å, indicating stable ligand-protein complexes without structural disruption. These findings indicate that longan peel is a promising natural source of larvicidal agents, providing a basis for the development of environmentally friendly, plant-derived inhibitors targeting *Aedes aegypti* larvae.

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