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Effects of quercetin and sabinene on antioxidant and detoxification enzymes of model pest *Drosophila melanogaster* (Diptera: Drosophilidae): Molecular docking investigation

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Keywords
Model insect, detoxification, molecular docking, quercetin, antioxidant enzymes

Abstract: In this study, molecular interactions of quercetin and sabinene - two naturally occurring compounds - with key antioxidant and detoxification enzymes were investigated in *Drosophila melanogaster*, a model organism in biological research. In the present study, the binding affinities of quercetin and sabinene with enzymes such as cytochrome P450, glutathione S-transferase, superoxide dismutase, catalase and glutamate-cysteine ligase were evaluated using molecular docking techniques. The results revealed that quercetin exhibits stronger interactions with all enzymes compared to sabinene, with the highest binding energy observed between quercetin and catalase (-10.7 kcal/mol). These findings suggest that quercetin significantly affects the antioxidant and detoxification systems of *D. melanogaster*, potentially enhancing oxidative stress. Sabinene demonstrated weaker binding across all enzymes. The study contributes valuable insights into the potential of quercetin and sabinene as agents in pest control strategies by targeting insect biochemical pathways.

Kuersetin ve sabinenin model böcek *Drosophila melanogaster*'in (Diptera: Drosophilidae) antioksidan ve detoksifikasyon enzimleri üzerindeki etkileri: Moleküler kenetlenme araştırması

Keywords
Model böcek, detoksifikasyon, moleküler yerleştirme, kuersetin, antioksidan enzimler

Öz: Bu çalışmada, biyolojik araştırmalarda model organizma olarak kullanılan *Drosophila melanogaster* üzerinde, iki doğal bileşik olan kuersetin ve sabinenin, önemli antioksidan ve detoksifikasyon enzimleri ile moleküler etkileşimleri araştırıldı. Bu çalışmada, kuersetin ve sabinenin sitokrom P450, glutatyon S-transferaz, süperoksit dismutaz, katalaz ve glutamat-sistein ligaz gibi enzimlerle bağlanma afiniteleri moleküler yerleştirme teknikleri kullanılarak değerlendirildi. Sonuçlar, kuersetin'in sabinene kıyasla tüm enzimlerle daha güçlü etkileşimler sergilediğini, kuersetin ve katalaz (-10,7 kcal/mol) arasında en yüksek bağlanma enerjisinin gözlemlendiğini ortaya koydu. Kuersetin'in *D. melanogaster* 'in antioksidan ve detoksifikasyon sistemlerini önemli ölçüde etkilediğini ve potansiyel olarak oksidatif stresi arttırabileceği belirlendi. Sabinen, tüm enzimlerde daha zayıf bağlanma afinitesi gösterdi. Bu çalışma, kuersetin ve sabinenin zararlı kontrol stratejilerinde ajan olarak potansiyeline ilişkin önemli sonuçlar sunmaktadır.

1. Introduction

Agricultural pest insects are a major cause of economic losses due to their damaging impact on crops. Among these, insect species within the Lepidoptera and Diptera order are particularly detrimental to agricultural regions. Consequently, there has been an increased focus on both chemical and biological control methods targeting these pests [1–5]. To mitigate these losses and enhance crop yields, the application of insecticides against these pests has become more prevalent. However, the

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escalation in the use of agricultural insecticides presents significant risks to non-target species and the broader environment. The toxic nature of these chemicals poses substantial threats to ecological balance and biodiversity. Therefore, it is critical to prioritize the use of insecticides with reduced toxicity in pest management strategies. Additionally, a comprehensive understanding of the physiology, biochemistry, and nutritional ecology of insect pests is essential for effective management [6, 7].

Drosophila melanogaster, commonly known as the fruit fly, has long been established as a pivotal model organism in the field of biological research [8]. Its significance stems from several advantageous characteristics, including its short life cycle, ease of maintenance in laboratory conditions, and well-mapped genome. As a model insect, *D. melanogaster* has been instrumental in advancing our understanding of fundamental biological processes, including genetics, development, and behavior. The fruit fly's genetic tractability allows for precise manipulation of genes, enabling researchers to investigate the roles of specific genes in various physiological and pathological conditions. Furthermore, the conservation of many biological pathways between *D. melanogaster* and higher organisms, including humans, underscores its utility in studying complex biological phenomena and disease mechanisms [9]. Consequently, *D. melanogaster* continues to be an invaluable tool in both basic and applied research, contributing to significant scientific breakthroughs across multiple disciplines [10, 11].

Organisms rely on their detoxification capacities to defend against the detrimental effects of environmental stressors [12]. The cytochrome P450 (CYP450) enzyme family is a multifunctional oxidase system that plays critical roles in the metabolism of both endogenous and exogenous compounds in insects. These enzymes, particularly involved in the detoxification and biotransformation of environmental xenobiotics such as pesticides, enable insects to adapt to chemical stress factors [13, 14]. The genetic diversity of CYP450 enzymes is a key factor in the development of resistance to different classes of pesticides, a phenomenon commonly observed in agricultural pests [15]. In addition, these enzymes participate in the biosynthesis of steroid hormones and pheromones, contributing to the regulation of developmental processes and reproductive behaviors in insects. Thus, the function of CYPP450 enzymes is essential for both defense against environmental stressors and the maintenance of physiological homeostasis in insects [16]. Glutathione S-transferase (GST), an enzyme implicated in both insecticide resistance and the detoxification of xenobiotics, also functions as an antioxidant through its peroxidase-like activity [17]. In both invertebrates and vertebrates, GST enzymes form a crucial part of a multifunctional detoxification system [18]. These enzymes play a key role in the phase II detoxification pathway, where they mitigate the toxic effects of electrophilic compounds [19]. This is achieved by conjugating xenobiotics with glutathione, thereby transforming them into less harmful forms through the metabolism of reactive intermediates produced by microsomal oxidation [20]. Superoxide dismutase (SOD) and catalase (CAT) are two key antioxidant enzymes that play crucial roles in cellular defense mechanisms. SOD mitigates the adverse effects of oxidative stress by converting the superoxide anion (O_2^-), a type of reactive oxygen species (ROS), into hydrogen peroxide (H_2O_2) and oxygen [21]. Since hydrogen peroxide can accumulate and become toxic within the cell, it is subsequently broken down into water and oxygen by the catalase enzyme. These two enzymes work synergistically to prevent cellular damage caused by oxidative stress [22].

SOD is predominantly found in mitochondria and cytosol, while catalase is primarily localized in peroxisomes. The activity of these enzymes is closely associated with various pathological processes, including aging, neurodegenerative diseases, and cancer. Therefore, the functions of SOD and CAT are critical for maintaining intracellular redox homeostasis [23]. Glutamate-cysteine ligase (GCL) is an important component of the cellular antioxidant defense mechanism in insects. GCL catalyzes the conjugation of the amino acids glutamate and cysteine to form γ-glutamylcysteine, which represents the first step in glutathione synthesis [24]. In insects, GCL activity increases under oxidative stress, helping to maintain cellular redox balance. The regulation of glutathione levels plays a critical role in the development of resistance to environmental stressors in insects. GCL and glutathione metabolism are key components of adaptive response mechanisms in insects exposed to pesticides and other toxic substances [25–27].

The aim of this study is to investigate the molecular interactions of two naturally occurring compounds, quercetin and sabinene, with key antioxidant and detoxification enzymes in model pest *D. melanogaster*.

2. Materials and Methods

In the present study, Autodock Vina [28] was used to perform a molecular docking study on target proteins to predict the interactions of quercetin and sabinene on antioxidant and detoxification enzymes. The sequences of the CYP450 (Q01603), GST (P41043), SOD (Q7JR71), CAT (P17336) and GLC (Q9W3K5) were taken from UniProt (https://www.uniprot.org/). Protein structure models were made in the Phyre2 [29]. Water molecules were removed from the antioxidant and detoxification enzyme structures.

Table 1. The docking results of quercetin on antioxidant and detoxification enzymes of *D. melanogaster*

Compound	Enzyme	Amino Acid	Interactions	ΔG (kcal/mol)
Quercetin	Ctyp450	PHE108; ARG372; ASP76	Conventional hydrogen bonds	-9.2
		PHE215	Pi-Pi stacked	
		ARG106; PHE215	Pi-cation	
		GLU374	Pi-anion	
	GST	GLN109; ILE111	Conventional hydrogen bonds	-8.5
		ILE111	Pi-alkyl	
		ARG145	Pi-cation	
		ASP143; ASP139	Pi-anion	
		ASN142	Pi-donor hydrogen bonds	
		GLN109	Unfavorable donor-donor	
		ASP143; ASP139	Attractive charge	
	SOD	GLU48; GLN68; LYS67	Conventional hydrogen bonds	-6.8
		THR136	Carbon hydrogen bonds	
		LYS67	Pi-alkyl	
		LYS67	Pi-cation	
		ALA61	Pi-sigma	
	CAT	ILE69; ARG170	Conventional hydrogen bonds	-10.7
		ARG68	Pi-alkyl	
		ASP389	Pi-anion	
		ASP389; GLU330	Attractive charge	
	GCL	ARG207; GLU328; ARG23	Conventional hydrogen bonds	-9.0
		LYS353; VAL350	Pi-alkyl	
		ARG23	Pi-cation	
		ASP417	Pi-anion	
		GLU20; ASP417	Attractive charge	
		VAL350	Pi-sigma	
		ARG16	Unfavorable acceptor- acceptor	

(CYP450: cytochrome P450; GST: glutathione S-transferase; SOD: superoxide dismutase; CAT: catalase; GCL: glutamate-cysteine ligase) (PHE: Phenylalanine; ARG: Arginine; ASP: Aspartic acid; GLU: Glutamic acid; GLN: Glutamine; ILE: Isoleucine; ASN: Asparagine; LYS: Lysine; THR: Threonine; ALA: Alanine; VAL: Valine)

Table 2. The docking results of sabinene on antioxidant and detoxification enzymes of *D. melanogaster*

Compound	Enzyme	Amino Acid	Interactions	ΔG (kcal/mol)
Sabinene	Ctyp450	PHE271; PHE447; PHE302; PHE137	Pi-alkyl	-6.7
		ILE184; ALA305	Alkyl	
	GST	TRP240; TYR75	Pi-alkyl	-5.1
		ALA69	Alkyl	
	SOD	VAL8; VAL148	Alkyl	-3.9
	CAT	PHE64; TYR358; PHE161	Pi-alkyl	-6.4
		MET61; ARG354; ALA357	Alkyl	
		TYR358	Pi-sigma	
	GCL	PRO465; PRO109; ARG468	Alkyl	-5.9

(CYP450: cytochrome P450; GST: glutathione S-transferase; SOD: superoxide dismutase; CAT: catalase; GCL: glutamate-cysteine ligase) (PHE: Phenylalanine; ARG: Arginine; ILE: Isoleucine; ALA: Alanine; VAL: Valine; TRP: Tryptophan; TRY: Tyrosine; MET: Methionine; PRO: Proline)

The missing polar hydrogens and Kollman charges on the crystal data were added. Inhibitors with the lowest energy insertion score were selected from the 10 conformations because of the calculation. Discovery Studio Visualizer (v-21.1.0.20298) was used to visualize the molecular docking results.

3. Results

Effects of quercetin on antioxidant and detoxification enzymes of *Drosophila melanogaster*

The interactions of quercetin with antioxidant and detoxification enzymes and the thermodynamic evaluation of these interactions reveal specific binding energies for each enzyme (Table 1). The interaction

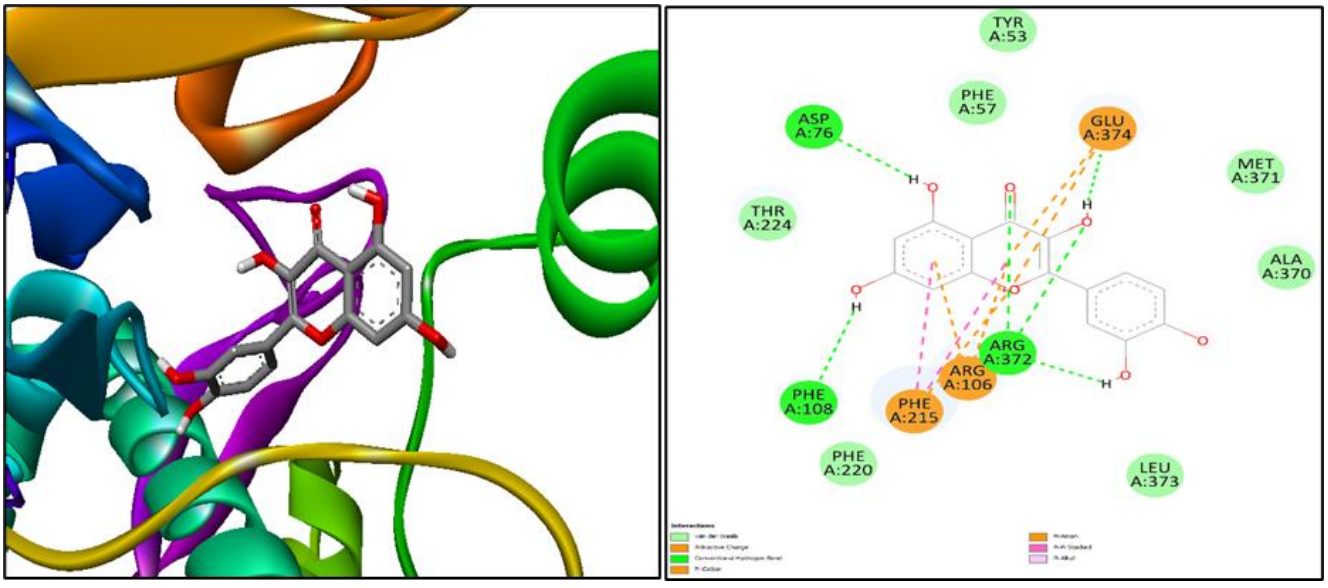

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Fig 1. The Molecular docking results of quercetin on the CYP450 in *D. melanogaster*. (a) Docking result of quercetin and CYP450. (b) 2D interactions of the quercetin with amino acids in the active site of the CYP450.

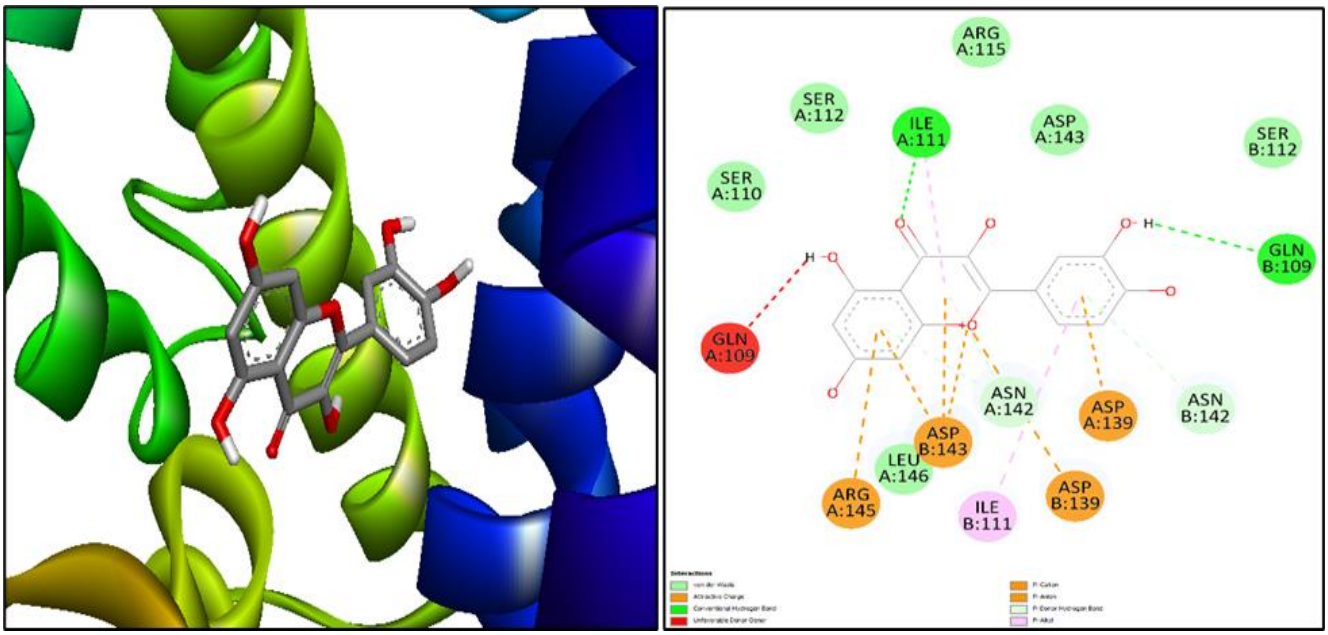

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Fig 2. The Molecular docking results of quercetin on the GST in *D. melanogaster*. (a) Docking result of quercetin and GST. (b) 2D interactions of the quercetin with amino acids in the active site of the GST.

between quercetin and Ctyp450 involves conventional hydrogen bonds with PHE108, ARG372, and ASP76 amino acids, Pi-Pi stacking with PHE215, and Pi-cation interactions between ARG106 and PHE215. These interactions are further supported by a Pi-anion bond with GLU374, and the binding free energy change (ΔG) is calculated as -9.2 kcal/mol (Fig 1).

In the interactions with GST, conventional hydrogen bonds are formed between GLN109 and ILE111, Pi-alkyl interactions with ILE111, and Pi-cation bonding with ARG145. Additionally, ASP143 and ASP139 exhibit Pi-anion and attractive charge interactions. However, an unfavorable donor-donor interaction is observed at GLN109 (Fig 2).

The binding energy of this complex is calculated as -8.5 kcal/mol. For the SOD enzyme, interactions involve conventional hydrogen bonds with GLU48, GLN68, and LYS67; carbon-hydrogen bonds with THR136; Pi-alkyl and Pi-cation interactions with LYS67; and Pi-sigma bonding with ALA61. As a result, the binding energy is determined to be -6.8 kcal/mol (Fig3). The interaction between quercetin and CAT is characterized by conventional hydrogen bonds with ILE69 and ARG170, Pi-alkyl bonds with ARG68, and Pi-anion interactions with ASP389.

Furthermore, attractive charge interactions are observed between ASP389 and GLU330 amino acids, with a calculated binding energy of -10.7 kcal/mol (Fig 4).

Lastly, in the interactions with GCL, conventional hydrogen bonds are formed with ARG207, GLU328, and ARG23; Pi-alkyl bonds with LYS353 and VAL350; Pi-cation interaction with ARG23; and Pi-anion bonding with ASP417.
Additionally, attractive charge interactions occur between GLU20 and ASP417, though an unfavorable acceptor-acceptor interaction is reported at ARG16. The binding energy of this complex is -9.0 kcal/mol (Fig 5).

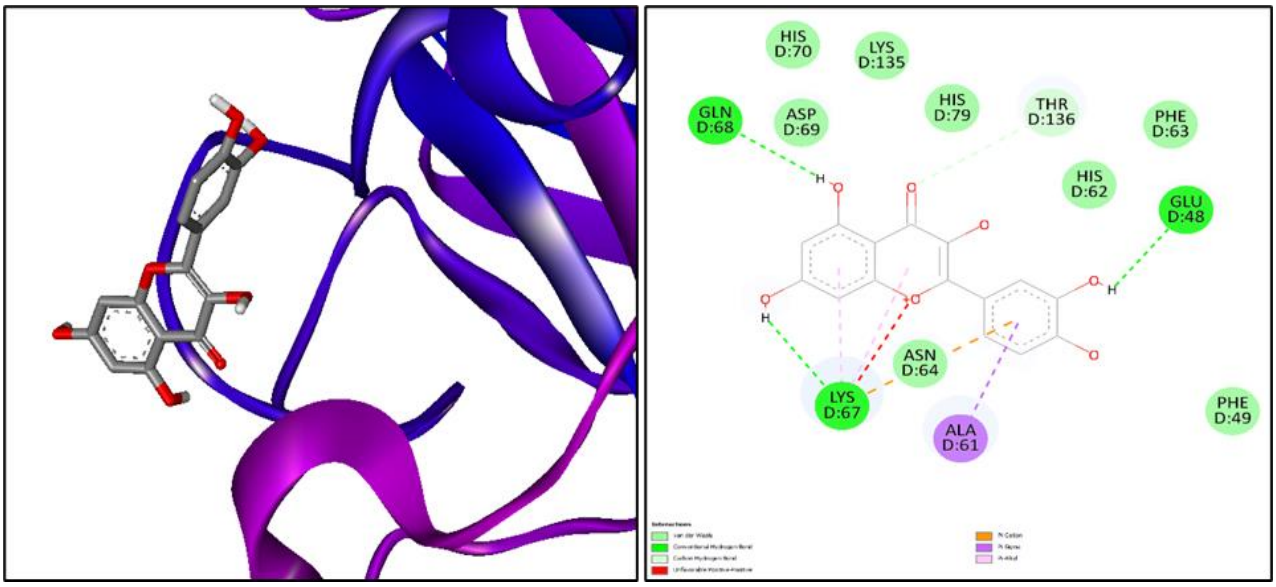

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Fig 3. The Molecular docking results of quercetin on the SOD in *D. melanogaster*. (a) Docking result of quercetin and SOD. (b) 2D interactions of the quercetin with amino acids in the active site of the SOD.

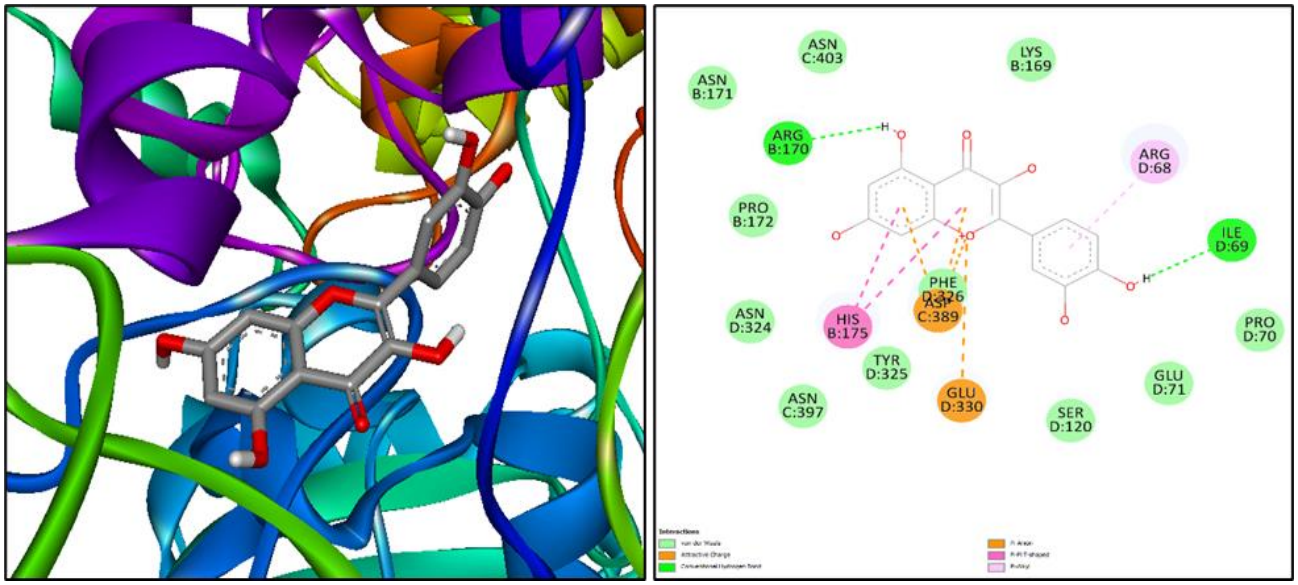

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Fig 4. The Molecular docking results of quercetin on the CAT in *D. melanogaster*. (a) Docking result of quercetin and CAT. (b) 2D interactions of the quercetin with amino acids in the active site of the CAT.

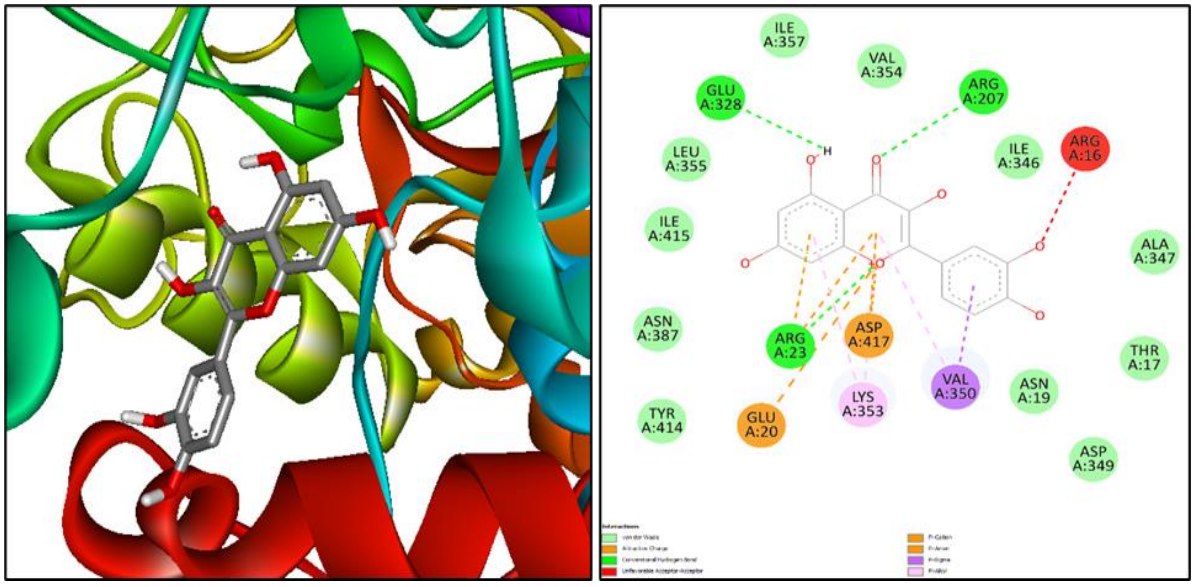

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Fig 5. The Molecular docking results of quercetin on the GCL in *D. melanogaster*. (a) Docking result of quercetin and GCL. (b) 2D interactions of the quercetin with amino acids in the active site of the GCL.

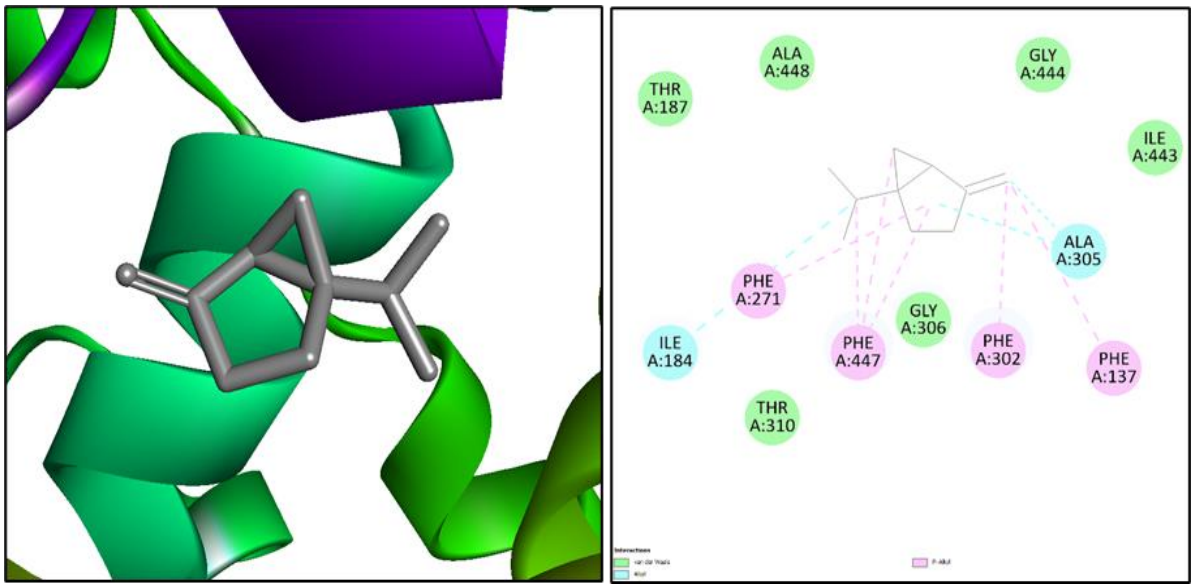

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Fig 6. The Molecular docking results of sabinene on the CYP450 in D. melanogaster. (a) Docking result of sabinene and CYP450. (b) 2D interactions of the sabinene with amino acids in the active site of CYP450.

Effects of sabinene on antioxidant and detoxification enzymes of *Drosophila melanogaster*

The interactions between sabinene and various enzymes have been studied, revealing specific binding energies (ΔG) for each enzyme (Table 2).

Sabinene interacts with Ctyp450 through Pi-alkyl interactions involving PHE271, PHE447, PHE302, and PHE137, along with alkyl interactions with ILE184 and ALA305. The binding free energy change for this complex is calculated as -6.7 kcal/mol (Fig 6).

In the case of GST, Pi-alkyl interactions are observed with TRP240 and TYR75, while alkyl interactions

occur with ALA69. The binding energy for this interaction is determined to be -5.1 kcal/mol (Fig 7).

For SOD, sabinene forms alkyl interactions with VAL8 and VAL148, resulting in a binding energy of -3.9 kcal/mol (Fig 8).

The interaction of sabinene with CAT involves Pi-alkyl interactions with PHE64, TYR358, and PHE161, alkyl interactions with MET61, ARG354, and ALA357, and a Pi-sigma bond with TYR358 (Fig 9).

The binding energy for this complex is -6.4 kcal/mol. Lastly, sabinene interacts with GCL through alkyl interactions involving PRO465, PRO109, and ARG468, with a binding energy of -5.9 kcal/mol (Fig 10).

4. Discussion

Although insecticides used in management of pest insects are effective, they can have negative impacts on the environment. These chemical substances contaminate soil, water sources, and air, leading to environmental pollution. Intensive use of insecticides in agriculture not only affects the targeted pests but also harms beneficial insects and other organisms, disrupting the ecological balance. Additionally, residues may enter the food chain, posing risks to human health. Therefore, the use of insecticides should be carefully managed, and environmentally friendly alternatives should be prioritized [30, 31, 32]. In the present study provides information about the effects of quercetin and sabinene on antioxidant and detoxification enzymes of model pest *D. melanogaster*. The findings showed that the antioxidant defense system of *D. melanogaster* was adversely affected by quercetin and sabinene.

Flavonoids are secondary metabolites produced by plants and exhibit oxidative stress-inducing properties on agricultural pest insects [33, 34, 35]. These compounds interfere with the biochemical processes of insects, leading to the accumulation of reactive oxygen species, which causes oxidative damage to cellular membranes, proteins, and DNA. Although insects activate their antioxidant defense systems to counterbalance this oxidative stress, the intensity and persistence of flavonoids can limit the effectiveness of these mechanisms. Consequently, flavonoids are considered potential agents in biological control, as they can increase the mortality rate of pest insects through oxidative stress pathways [36]. In the present study, it was determined that quercetin established a strong interaction with *D. melanogaster* antioxidant and detoxification enzymes such as SOD, CAT, GLC, CTYP450 and GST. In another previous study, it was reported that the amount of ROS increased in *D. melanogaster* exposed to Trichloroethylene and CAT, ACHE and GST activities were adversely affected [37]. In another study, it was determined that cell damage occurred in model insect *Galleria mellonella* larvae exposed to Ni (II) p-hydroxybenzoate with caffeine and that the elimination of this damage increased the antioxidant response [38]. Güneş and Büyükgüzel [3] reported that the levels of oxidative stress indicators malondialdehyde and protein carbonyl in *D. melanogaster* larvae, pupae and adults exposed to boric acid increased depending on the concentration, and the level of antioxidant GST increased to eliminate oxidative damage. In another study, it was determined that SOD was inhibited and CAT and GST levels were induced in *D. melanogaster* exposed to Mancozeb, an ethylene-bis-dithiocarbamate containing manganese and zinc [39]. In another work, it was determined that strong interaction between Cucurbitacin, which have tetracyclic compounds, and CAT (- 10.6 kcal/mol affinity energy) of model insect *G. mellonella* [40]. The high binding energy between antioxidant enzymes and herbal compounds may indicate that these compounds have adverse effects on enzyme activities. In another study, the effects of organic extracts of *Solidago graminifolia* on *Spodoptera frugiperda* acetylcholinesterase levels were determined by molecular docking method.

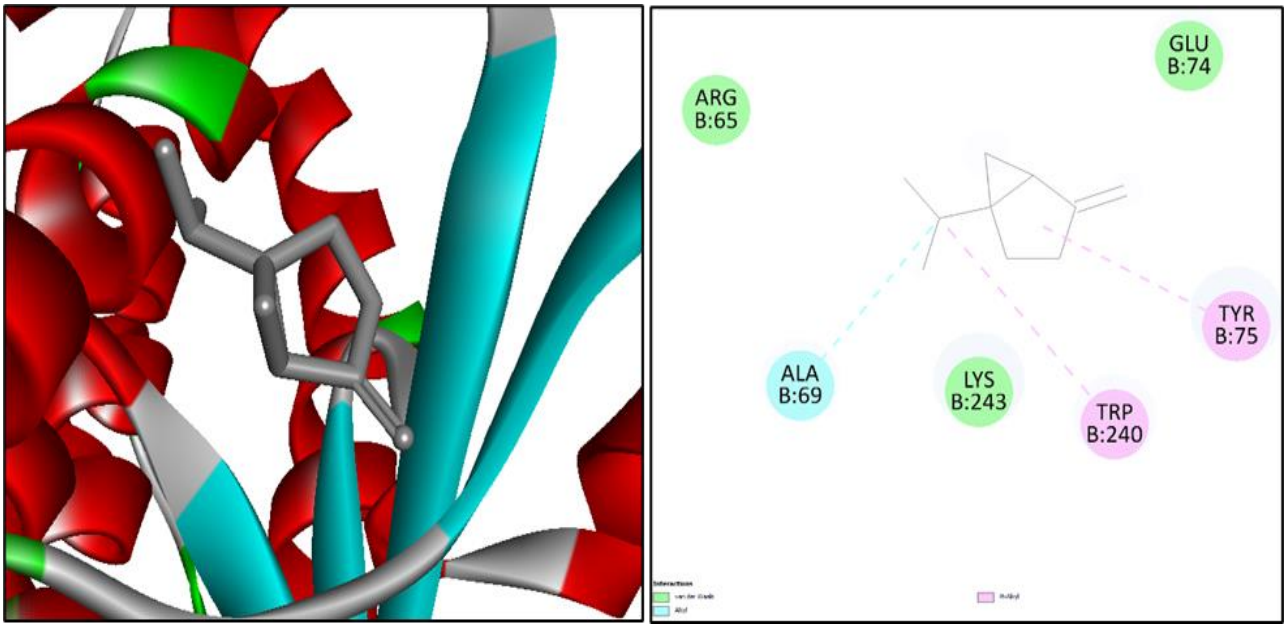

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Fig 7. The Molecular docking results of sabinene on the GST in *D. melanogaster*. (a) Docking result of sabinene and GST. (b) 2D interactions of the sabinene with amino acids in the active site of the GST.

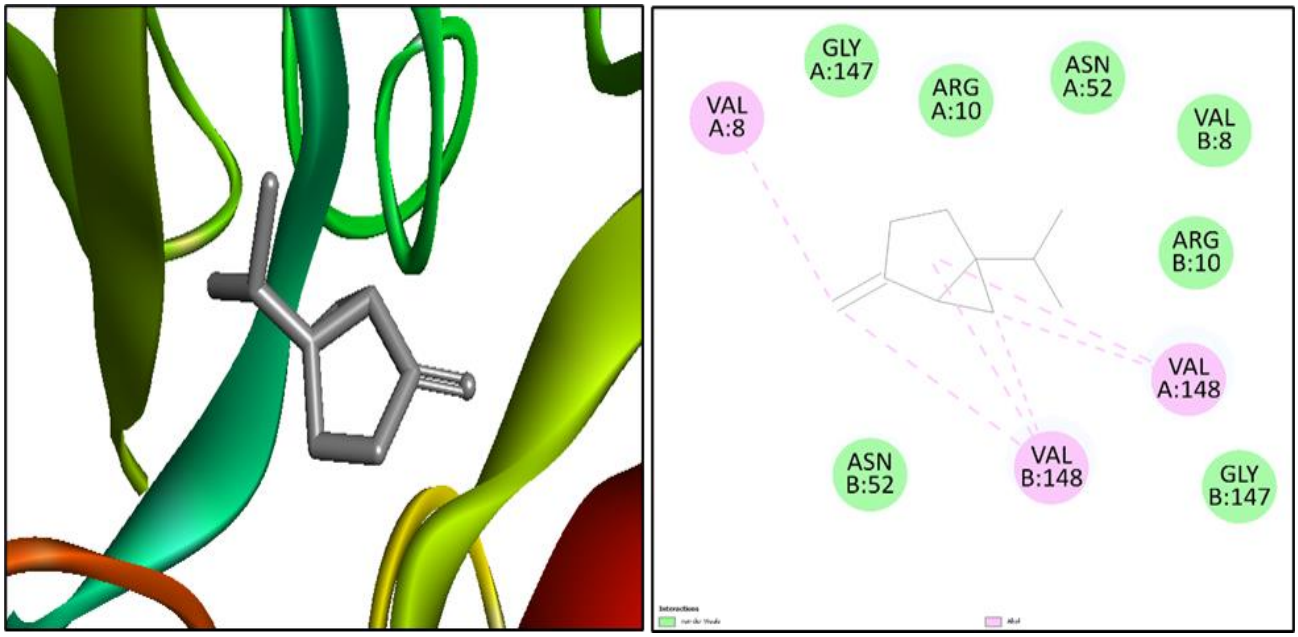

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Fig 8. The Molecular docking results of sabinene on the SOD in *D. melanogaster*. (a) Docking result of sabinene and SOD. (b) 2D interactions of the sabinene with amino acids in the active site of the SOD.

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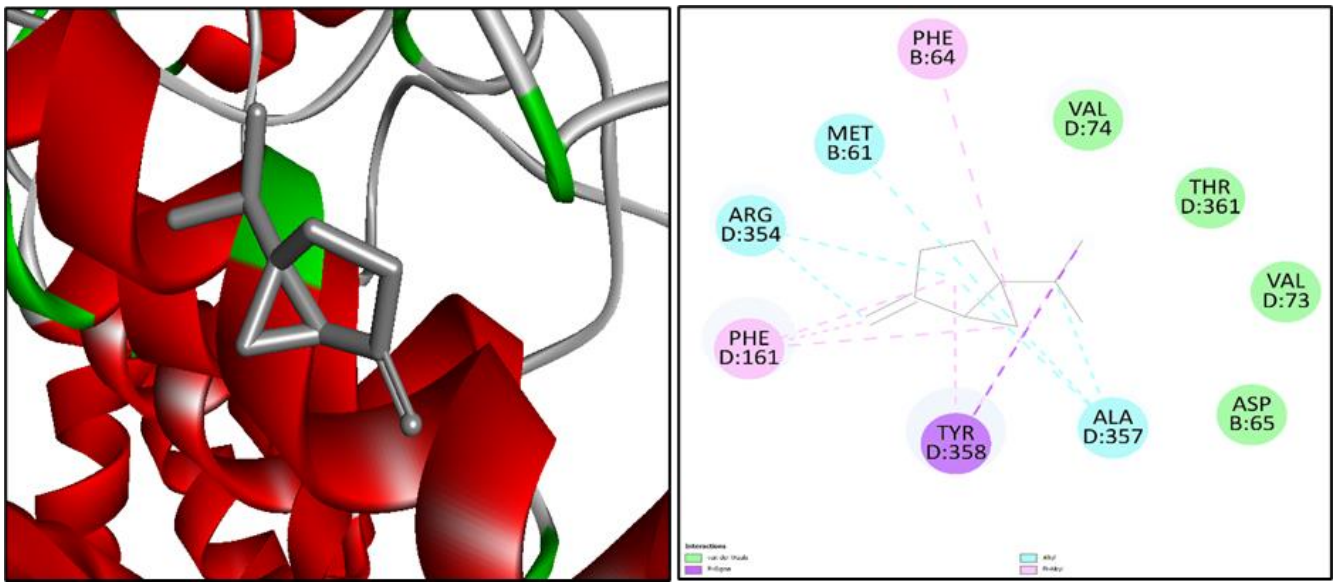

Fig 9. The Molecular docking results of sabinene on the CAT in *D. melanogaster*. (a) Docking result of sabinene and CAT. (b) 2D interactions of the sabinene with amino acids in the active site of the CAT.

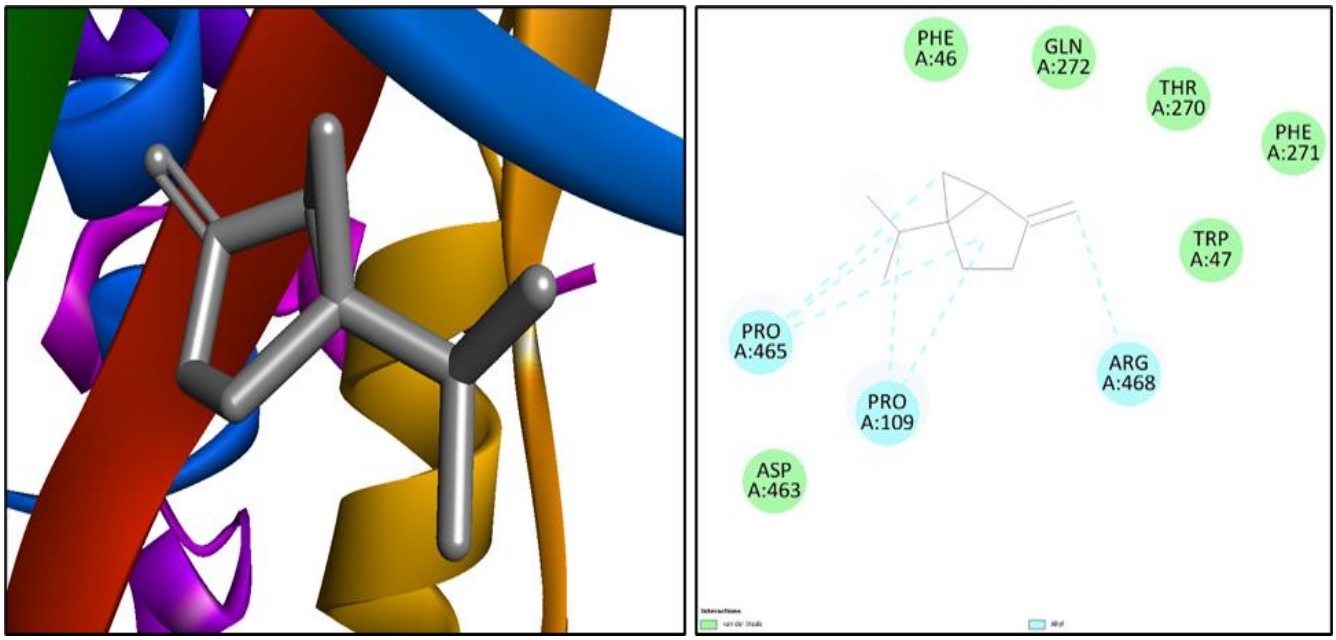

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Fig 10. The Molecular docking results of sabinene on the GCL in *D. melanogaster*. (a) Docking result of sabinene and GCL. (b) 2D interactions of the sabinene with amino acids in the active site of the GCL.

Molecular docking analysis of quercetin into the active site of *S. frugiperda* acetylcholinesterase showed a binding energy value of -5.4 kcal/mol [41]. Similarly, in this study, high binding energy of quercetin with antioxidant enzymes of *D. melanogaster* was measured. Sabinene had lower binding energy with *D. melanogaster* antioxidant enzymes.

In living organisms, cytochrome P450 and GST are important detoxification enzymes that play key roles in breaking down and eliminating harmful substances, such as toxins, pesticides, and drugs, helping to protect cells from damage [42]. In this study, it was determined that quercetin had strong binding with detoxification enzymes such as GST, CYT P450 and GLC. In another study, it was observed that detoxifying enzymes were inhibited in *Aedes aegypti* (Diptera: Culicidae) exposed to extracts obtained from *Piper betle* and *Sphaeranthus indicus* leaves. Another study tested the larval resistance of model insect *G. mellonella* to alkaloids from *Berberis microphylla*. The aim was to identify the enzymes involved in alkaloid detoxification through bioinformatic analysis and qRT-PCR. The findings showed that GST, CYT P450, carboxylesterases (CarE), rather than the enzymes involved in alkaloid detoxification, resulting from an increased metabolic mechanism [43]. In a previous study, GST levels were increased in model insect *D. melanogaster* exposed to dietary gemifloxacin [44]. In another study, it was determined that the antioxidant system was adversely affected in the model organism *G. mellonella* larvae exposed to copper oxide and titanium dioxide nanoparticles [45, 46]. Studies show that herbal and chemical agents have an effect on detoxification and antioxidant enzymes. In this study, it was determined that quercetin interacted with detoxification enzymes with strong binding energy, while there was a low interaction with sabinene.

5. Conclusion

In this study, the binding potential of *D. melanogaster* exposed to quercetin and sabinene with antioxidant and detoxification enzymes was investigated for the first time in silico. In silico studies provide important information in revealing the effects of many chemicals on model systems and their potential to be used as safe pesticides in agriculture. Further research is warranted to explore the practical application of these compounds as eco-friendly pesticides in agriculture.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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