

MOLECULAR DOCKING ANALYSIS OF SELECTED ESSENTIAL OIL CONSTITUENTS WITH CYTOCHROME P450 OF *PLUTELLA XYLOSTELLA*

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ABSTRACT

Plutella xylostella (L.) is a major pest of cruciferous crops and has developed resistance to several conventional insecticides, partly due to enhanced detoxification mechanisms involving mixed-function oxidases (MFOs), particularly cytochrome P450 enzymes. Essential oil (EO) constituent compounds are promising botanical insecticidal agents; however, their interactions with detoxification enzymes remains limited. The present study investigated the potential interactions of selected EO constituent compounds with cytochrome P450 of *P. xylostella* using an *in silico* molecular docking approach. The cytochrome P450 protein sequence (NCBI accession no. XP_082886576.1) was modelled using SWISS-MODEL and validated through Ramachandran plot analysis. The validated protein model was used for molecular docking with selected EO constituents using PyRx with AutoDock Vina, and ligand–protein interactions were subsequently analysed using BIOVIA Discovery Studio Visualizer. All tested compounds showed favourable binding with cytochrome P450, with binding affinities ranging from -5.2 to -6.2 kcal mol⁻¹. Among the tested compounds, citral exhibited the strongest predicted binding affinity (-6.2 kcal mol⁻¹), followed by eugenol (-5.9 kcal mol⁻¹), while D-limonene showed the weakest binding (-5.2 kcal mol⁻¹).

KEYWORDS: *Plutella xylostella*, cytochrome P450, molecular docking, binding affinity, Essential oil compounds.

INTRODUCTION

Plutella xylostella (L.) (Lepidoptera: Plutellidae), commonly known as the diamondback moth (DBM), is one of the most destructive insect pests of cruciferous crops worldwide (Paudel et al., 2022). Its short generation time, high reproductive potential, and continuous exposure to insecticides have contributed to the development of resistance to several groups of conventional insecticides, making its management increasingly difficult (Banazeer et al., 2022; Shehzad et al., 2023). Consequently, there is growing interest in safer and more sustainable alternatives, including essential oils (EOs) and their bioactive constituents. EOs are complex mixtures of volatile secondary metabolites, mainly comprising monoterpenes, sesquiterpenes, phenylpropanoids, and other aromatic compounds (Ben Miri, 2025). Several EO constituents have demonstrated insecticidal, repellent, antifeedant, and growth-inhibitory activities against DBM (Liu et al., 2022; Cao et al., 2019). Their insecticidal effects may involve interference with the nervous system, disruption of metabolic processes, inhibition of detoxification enzymes, and alteration of other physiological functions. Among the major detoxification enzymes in insects, mixed-function oxidases (MFOs), particularly cytochrome P450 monooxygenases, play an important role in the metabolism and detoxification of endogenous and exogenous compounds (Esteves et al., 2021; Chakraborty et al., 2023). Enhanced activity or expression of cytochrome P450 enzymes is also associated with the development of insecticide resistance in DBM. Therefore, interaction of botanical compounds with cytochrome P450 may provide important insights into their possible effects on insect detoxification mechanisms.

Molecular docking is a useful *in silico* approach for predicting the binding affinity and interaction patterns between bioactive compounds and target proteins (Agu et al., 2023; Agamah et al., 2020). It provides information on binding energies, interacting amino acid residues, and the types of molecular interactions involved, thereby helping to identify potential ligand–protein interactions at the molecular level. Such analysis

can complement experimental studies and assist in understanding the possible mechanisms underlying the insecticidal activity of EO constituents.

Despite the reported insecticidal potential of several EO constituents against DBM, information on their molecular interactions with detoxification enzymes such as MFOs remains limited. Therefore, the present study was undertaken to investigate the molecular interactions of selected EO constituent compounds with the cytochrome P450 enzyme of DBM using molecular docking. The study aimed to compare their predicted binding affinities, identify key amino acid residues involved in ligand binding, and characterize the major intermolecular interactions, thereby providing insights into their potential interaction with the detoxification machinery of DBM.

MATERIALS AND METHODS

EO constituent compounds

Eight EO compounds were used for docking studies. The list of EO constituent compounds selected for docking studies is presented in Table 1.

Ligand preparation

The three-dimensional structures of the selected EO constituent compounds were obtained from the PubChem database in 3 SDF format. The structures were converted to PDB format and imported into PyRx for ligand preparation. The ligands were energy-minimized using the Universal Force Field (UFF), and appropriate hydrogen atoms and partial charges were assigned. The prepared ligands were subsequently converted into the required docking format for molecular docking.

Macromolecule/protein preparation

The FASTA sequence of the target cytochrome P450 protein of DBM was retrieved from the NCBI Protein database using the accession number XP_082886576.1. The retrieved amino acid sequence was submitted to the SWISS-MODEL server for three-dimensional homology modelling. The resulting protein model was subsequently evaluated for structural quality using a Ramachandran plot. The model was considered suitable for subsequent molecular docking based on the distribution of residues in the favoured, allowed, and disallowed regions of the Ramachandran plot. The validated three-dimensional protein structure was then used as the receptor for molecular docking studies with the selected essential oil constituent compounds.

In silico molecular docking

Molecular docking was performed using PyRx software with AutoDock Vina to investigate the binding interactions between the selected essential oil constituent compounds and the modelled cytochrome P450 protein of DBM. The validated three-dimensional protein structure was imported into PyRx and prepared as the macromolecular receptor, while the selected EO constituents were prepared as ligands. The protein was kept rigid, whereas the ligands were treated as flexible during docking. A grid box was defined to encompass the selected binding region of the cytochrome P450 protein. AutoDock Vina was used to generate multiple binding poses for each ligand and to estimate their binding affinities. The docking poses were ranked according to their binding energy (kcal mol^{-1}), with more negative values indicating stronger predicted binding affinity.

RESULTS

Ramachandran plot analysis of modelled protein

The cytochrome P450 protein was modelled using SWISS-model and shown in Fig 1. Ramachandran plot analysis of the modelled cytochrome P450 protein showed that 96.52% of the residues were located in the favoured regions, 3.26% in the allowed regions, and only 0.22% in the disallowed region, indicating good stereochemical quality of the predicted protein model (Table 2, Fig 2).

In silico molecular docking

All tested compounds showed favourable binding with the modelled cytochrome P450 protein, with binding affinities ranging from -5.2 to $-6.2 \text{ kcal mol}^{-1}$ (Table 3). Among the tested compounds, citral exhibited the strongest predicted binding affinity ($-6.2 \text{ kcal mol}^{-1}$), followed by eugenol ($-5.9 \text{ kcal mol}^{-1}$). Eucalyptol and p-cymene showed comparable binding affinities of $-5.7 \text{ kcal mol}^{-1}$, whereas α -pinene, β -pinene, and γ -terpineol exhibited binding affinities of $-5.3 \text{ kcal mol}^{-1}$. D-limonene showed the weakest binding affinity ($-5.2 \text{ kcal mol}^{-1}$) among the tested compounds. The 2D and 3D docking structures are depicted in Fig 3.

DISCUSSION

The present molecular docking analysis demonstrated that the selected EO constituent compounds exhibited favourable interactions with the modelled cytochrome P450 of DBM, with predicted binding affinities ranging from -5.2 to $-6.2 \text{ kcal mol}^{-1}$. The variation in binding affinity among the compounds indicates differences in their ability to interact with the cytochrome P450 binding region, which may be related to differences in their molecular structure, functional groups, and hydrophobicity. Among the tested compounds, citral showed the strongest predicted binding affinity ($-6.2 \text{ kcal mol}^{-1}$), followed by eugenol ($-5.9 \text{ kcal mol}^{-1}$). These results suggest that both compounds may have a comparatively greater propensity to interact with the target protein than the other tested constituents. Similarly, Gaire et al. (2021) reported that EOs inhibited cytochrome P450 activity in deltamethrin-resistant bed bug populations, supporting the potential of EOs to interfere with insect detoxification mechanisms. Moustafa et al. (2023) provided further evidence for the interaction of citral with

cytochrome P450, reporting that citral formed interactions with cysteine (CYS345) and histidine (HIS343) residues of the enzyme. The authors suggested that interaction with cytochrome P450 may represent one of the mechanisms underlying the insecticidal activity of *Cymbopogon citratus* EO and citral against *Spodoptera littoralis*. Likewise, several studies have shown inhibition and modulation of detoxification enzymes by plant EOs and their components (Rizvi et al., 2018; Chauhan et al., 2022; Abdelaal et al., 2021).

CONCLUSION

The molecular docking analysis demonstrated that the selected EO constituent compounds exhibited favourable interactions with cytochrome P450 of DBM. Among the tested compounds, citral showed the strongest predicted binding affinity ($-6.2 \text{ kcal mol}^{-1}$), followed by eugenol ($-5.9 \text{ kcal mol}^{-1}$), and both formed multiple interactions with key amino acid residues of the target protein. These findings suggest that citral and eugenol may have greater potential to interact with the cytochrome P450-mediated detoxification system of DBM.

Authors contribution

KS conducted the extensive literature review and contributed to the conceptualization, methodology, validation, formal analysis, investigation, and preparation of the original draft. TA, MM, and SV assisted with literature collection, provided supervision and resources, and reviewed the original draft. SA, SVK and PM contributed substantially to manuscript drafting and critical revision. All authors have read and approved the final version of the manuscript.

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Declarations

Consent for Publication We confirm that the manuscript has been read and approved by all named authors.

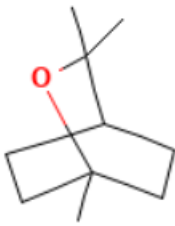
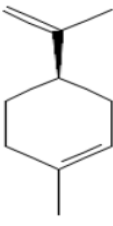
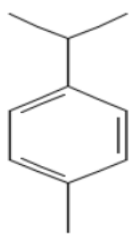
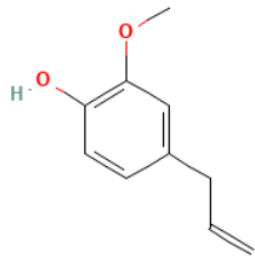
Conflict of Interest The authors declare no conflict of interest.

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Table 1. List of EO constituent compounds selected for molecular docking

EO compound	constituent	PubChem ID	Structure
Eucalyptol		2758	
D-Limonene		440917	
P-cymene		7463	
Eugenol		3314	

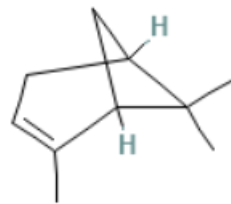
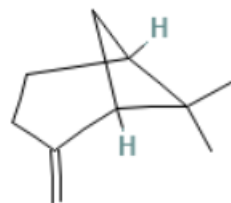
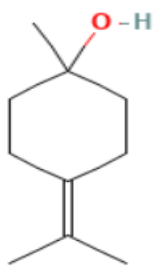
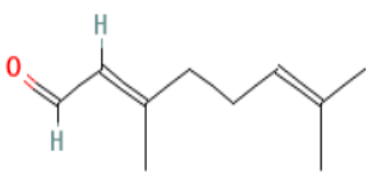
Alpha-pinene	6654	
Beta-pinene	14896	
Gamma-Terpineol	11467	
Citral	638011	

Table 2. Ramachandran plot statistics of modelled Cytochrome P450 protein of *Plutella xylostella*

Ramachandran category	No. Of residues	Percentage (%)
Favoured regions	444	96.52
Allowed regions	15	3.26
Disallowed regions	1	0.21
Total	460	100

Table 3. Molecular docking results showing interactions between EO constituent compounds and cytochrome P450 of *Plutella xylostella*

EO constituent compound	Binding affinity (Kcal/mol)	Binding residues	Binding interactions
Eucalyptol	-5.7	HIS248, TYR251, HIS247, ARG113, LEU112, PHE115, LYS244	Van der Waals, alkyl, pi-alkyl

D-Limonene	-5.2	PHE123, ILE372, VAL122, ARG113, PHE213	MET219, ALA304, PHE109,	Van der Waals, pi-sigma, alkyl, pi-alkyl
P-cymene	-5.7	PHE123, ALA304, MET219, PHE214, ARG113	ILE372, VAL122, PHE109,	Van der Waals, pi-sigma, pi-pi T shaped, pi-sulfur, pi-pi stacked, alkyl, pi- alkyl
Eugenol	-5.9	VAL217, LEU243, MET219, PHE109, HIS247, ALA304	SER213, ARG113, PHE214, PHE123, VAL122,	Van der Waals, pi-pi T shaped, Carbon Hydrogen Bond, pi-pi stacked, alkyl, pi-alkyl
Alpha-pinene	-5.3	HIS374, LEU96, ALA368, GLY434, ARG439, LEU395, ARG438	ARG104, ILE372, GLY371, LEU397, SER435, PHE433,	Van der Waals, alkyl
Beta-pinene	-5.3	PHE115, HIS248, LYS244, HIS247	TYR251, LEU112,	Van der Waals, alkyl, pi- alkyl
Gamma-Terpineol	-5.3	ARG113, PHE123, VAL122, ALA368, ILE372, VAL367	PHE214, GLN303, MET219, THR308, ALA304,	Van der Waals, alkyl, pi- alkyl
Citral	-6.2	HIS247, MET250, PHE109, PHE214, PHE123, ILE372, ASP300	ARG113, SER213, GLN303, MET219, VAL122, ALA304,	Van der Waals, alkyl, pi- alkyl, conventional hydrogen bond, pi-sigma

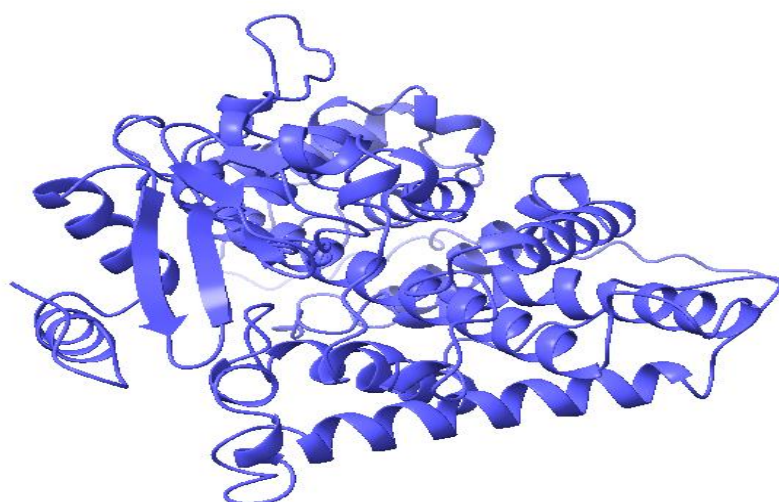


Fig 1. modelled protein of Cytochrome P450

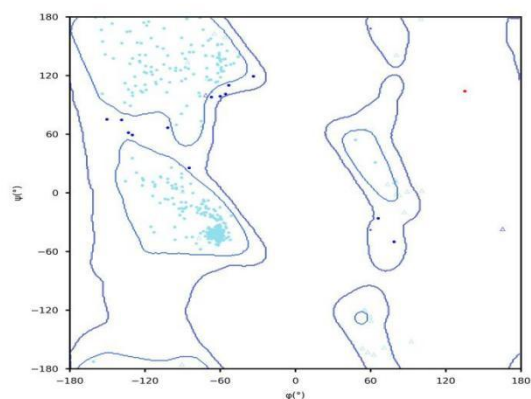
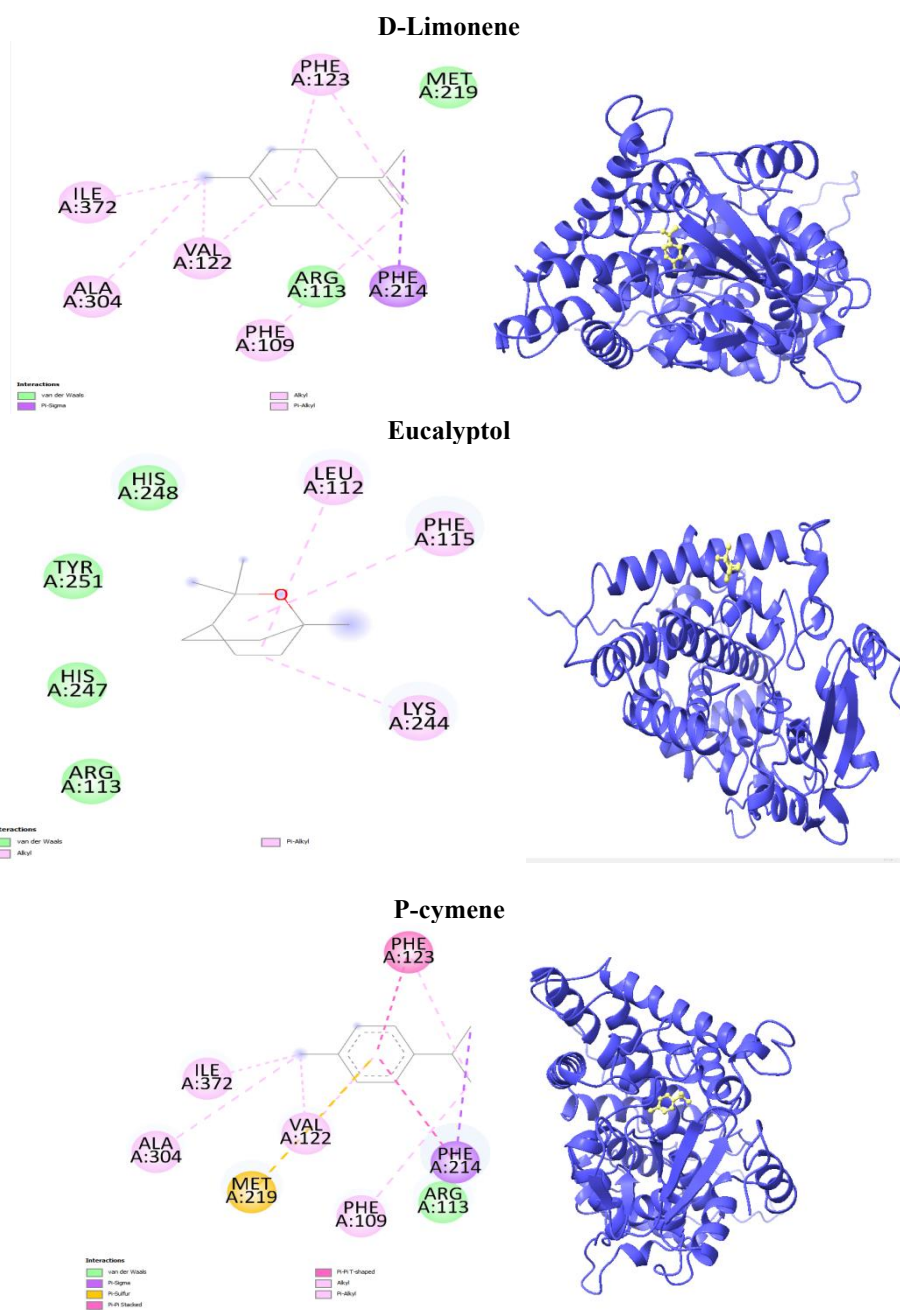
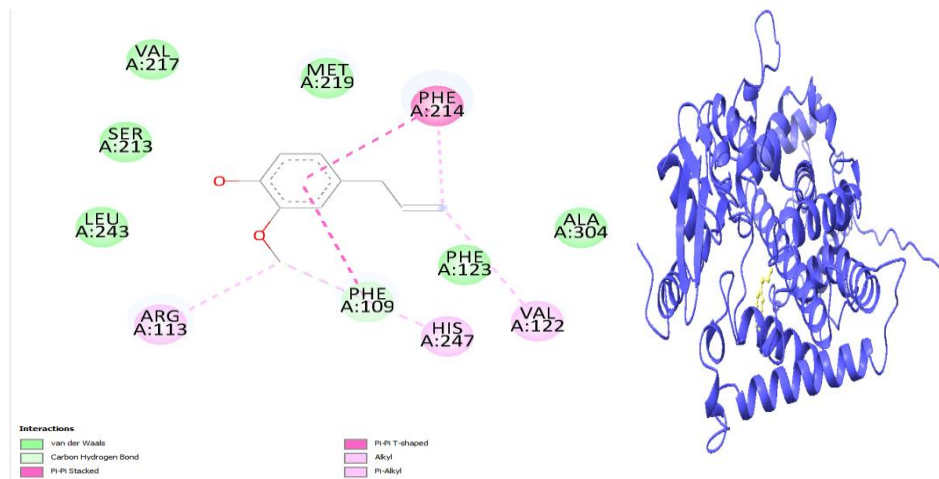


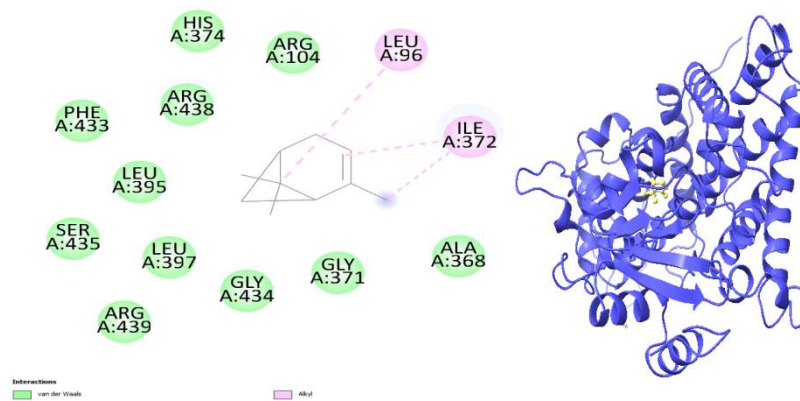
Fig 2. Ramachandran plot analysis of Cytochrome P450 protein of *Plutella xylostella* (cyan, blue and red (dots/triangles) represent torsion angles of favoured, allowed and disallowed regions respectively; dot represents residues other than glycine and triangles represents glycine)



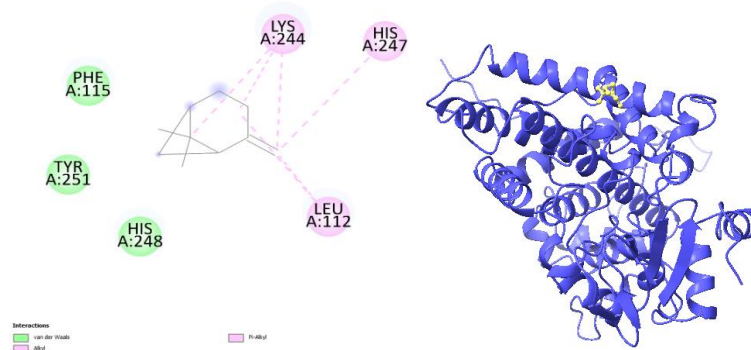
Eugenol



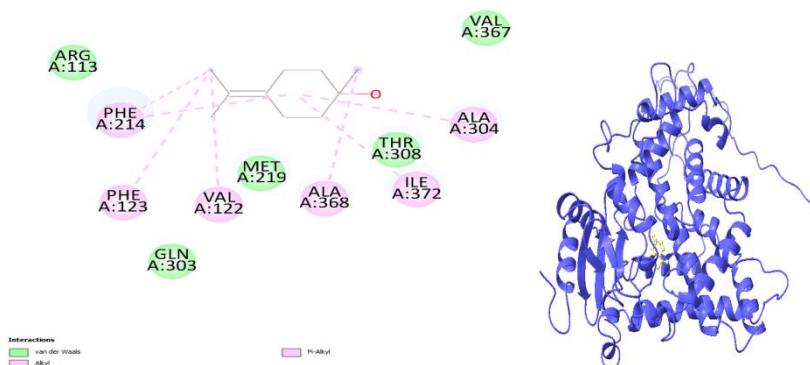
Alpha-pinene



Beta-pinene



Gamma-terpineol



Citral

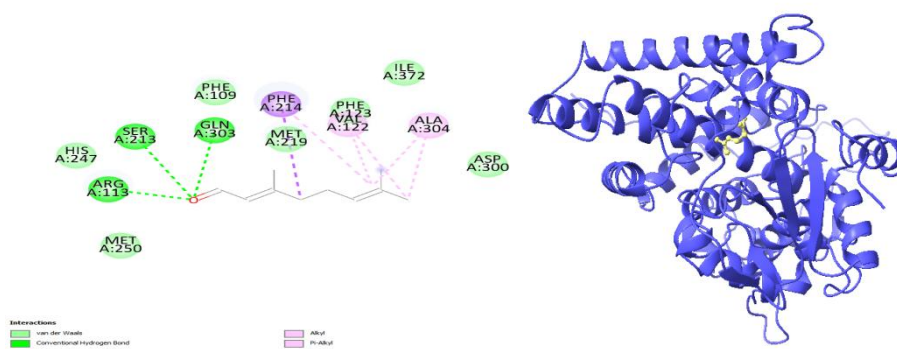


Fig 3. 2D and 3D docking interactions of EO constituent compounds and Cytochrome P450 of *Plutella xylostella*