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*Original Research Article*

# Physicochemical Properties, Antimicrobial and Insecticidal Activities of Carvacrol: *In Vitro* and *In Silico* Toxicity Studies

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ABSTRACT

Carvacrol **is** a phenolic compound found in the essential oils of aromatic plants. Carvacrol is known for its numerous biological activities. This study aimed to evaluate the antimicrobial, and insecticidal properties of carvacrol *in vitro* and *in silico*. Antimicrobial activity was determined using broth microdilution method. Insecticidal activity was evaluated against adult *Callosobruchus maculatus* using the fumigant toxicity bioassay. *In silico* molecular docking against selected target proteins were done to theoretically assess carvacrol's potential antimicrobial and insecticidal activities. ADME/Toxicity prediction was also conducted. Carvacrol exhibited potent antimicrobial activity with minimum inhibitory concentrations (MICs) of 4.125% against *Escherichia coli* and *Staphylococcus aureus*, 2.06% against *Listeria innocua* and *Pseudomonas aeruginosa*, 2.06% against *Aspergillus niger* and *Penicillium digitatum*, and 4.125% against *Candida glabrata*. The minimum bactericidal concentration (MBC) ranged from 8.75 to 16.5%, while the minimum fungicidal concentration (MFC) was ≥ 33.00%. Carvacrol demonstrated notable insecticidal effect against adult *Callosobruchus maculatus*, resulting in 100% mortality at 20 µL/L of air after 12 hours of exposure. The ovicidal impact was significant, with complete inhibition of egg-laying at 20 µL/L of air. Carvacrol also completely inhibited adult emergence, confirming its toxicity against *C. maculatus*. *In silico* analysis revealed that carvacrol exhibited potent activity against *E. coli*, *S. aureus*, and *A. niger* with glide scores of -6.516, -4.905, and -5.321 kcal/mol, respectively. Carvacrol significantly inhibited acetylcholinesterase and chitin synthase, with glide scores of -6.747 and -5.442 kcal/mol, respectively. These results suggest that carvacrol is a promising natural alternative to synthetic pesticides and antimicrobial agents.

***Keywords:*** *Carvacrol, Toxicity, Bacteria, Fungi, Insects, In vitro, In silico*

## Introduction

Carvacrol is renowned for its numerous biological activities. In addition to its antimicrobial properties against various pathogenic bacteria and fungi, it exhibits anti-inflammatory, antioxidant, and anticancer effects.[7–9].These properties make it particularly appealing microorganisms and insects.[8,10–12] This study evaluated carvacrol's antimicrobial and insecticidal activity against *Callosobruchus maculatus*, a major pest of stored legumes. The economic losses caused by this insect are significant, and current control methods, often relying on synthetic insecticides, have drawbacks such as increased insect resistance and risks to human health and the environment.[13,14]. Scientific research on plant-based products has focused on the phytochemical analysis and bioactivity of various plant extracts at the laboratory scale. In the present study, we aim to highlight the importance of a bioactive molecule of plant origin in the medical and agricultural fields. In this context, we examined the insecticidal, antibacterial, and antifungal activities of carvacrol, its mechanisms of action, its efficacy compared to conventional synthetic products, and its potential for integration into sustainable pest management strategies were also evaluated. The findings from this study could pave the way for new biocontrol approaches based on natural compounds, thereby reducing the over dependence on synthetic products, and ultimately contributing to food security, health and environmental protection.

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## Materials and Methods

### *Chemical*

Analytical grade carvacrol was obtained from Sigma-Aldrich (St. Louis, MO, USA), and used without further purification.

### *Determination of antimicrobial activity*

#### *Microbial strains*

The bacterial strains used in this study include *Pseudomonas aeruginosa* (ATCC 15442), *Listeria innocua* (ATCC 33090), *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (ATCC

10536). The fungal strains include *Aspergillus niger*, *Candida glabrata*, and *Penicillium digitatum*. All microbial strains were provided by the Laboratory of Bioresources, Biotechnology, Ethnopharmacology, and Health at the Faculty of Sciences, Oujda, Morocco.

*Evaluation of minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC)*

The MIC, MBC, and MFC of carvacrol was determined in 96-well microplates using broth microdilution method as described by [15] Carvacrol was tested at concentrations ranging from 33% to 0.128%. Microbial inocula were standardized for both bacteria and fungi, and 50 µL of the standardized inocula was added to each well. Tetracycline (1 mg/mL) and cycloheximide (1 mg/mL) were used as the positive controls for the bacterial strains and fungal strains, respectively. The plates were incubated at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungi. After the incubation period, 15 µL of 0.015% resazurin solution was added to each well, followed by an additional 2-hour incubation to assess metabolic activity. A colour change from blue (resazurin) to pink (resorufin) indicated microbial viability. Each test was conducted in triplicate to ensure precision and reproducibility. To determine MBC and MFC, samples from wells showing no visible growth were inoculated onto agar plates and incubated for 24 hours at the appropriate temperature. The lowest concentration at which no microbial growth was observed on the agar plates was recorded as the MBC or MFC.[15]

*Determination of insecticidal activity*
*Insect rearing*

*Callosobruchus maculatus* insects were obtained from an infested chickpea sample collected from legume wholesalers in Fez City. The insects were identified in the laboratory, and the seeds used for insect rearing were purchased from a local grocery store in Fez, northern Morocco. The seeds were stored in sealed polyethylene bags and kept in a freezer at -10°C for one week until use. Mass rearing of the insects was carried out in 1 L glass jars, maintained in a growth chamber at a temperature of 25 ± 1°C, with controlled relative humidity and a photoperiod of 14 hours light/10 hours dark. This process was repeated for three successive generations by placing 50 pairs of male and female beetles in jars containing 100 g of chickpea seeds. Female beetles were allowed to lay eggs on the seeds for 24 hours, after which they were removed. The seeds containing eggs were kept in the rearing chamber until adult emergence.

*Treatment with carvacrol*

The fumigant insecticidal activity of carvacrol was evaluated against adult *C. maculatus* at concentrations of 1, 5, 10, and 20 µL/L of air. The adult *C. maculatus* were exposed to the treatment (carvacrol) for 12, 24, 36, and 48 hours in 1 L jars containing 20 g of chickpea seeds. Jars without carvacrol were used as controls. Adult mortality was assessed after 12 hours. The treatment concentrations were determined based on preliminary experiments and included increasing concentrations of 0.5, 1, 5, 10, 15, 20, 30, 40, 60, and 80 µL/L of air. Dead insects were removed every 12 hours for 48 hours. After 13 days from the start of the experiment, the eggs laid on the grains were also counted. Additionally, the number of emerged insects was recorded 28 days after the experiment began. Mortality percentages were calculated using the Abbott's formula as shown below:

$$Pc = 100\ (P0-Pt)/100-Pt) \quad \text{Eq. 1}$$

Where;
Pc = corrected mortality rate in percentage (%), P0 = observed mortality in the test, and
Pt = observed mortality in the control.
The percentage reduction in the number of eggs and emerged adults at each concentration was calculated in comparison to the control using the following formula:

$$PR= ((NC-NT)/(NC*100) \quad \text{Eq. 2}$$

Where;
PR = percentage reduction in egg laying or emerged insects, NC = number of eggs or emerged insects in the control, and NT = number of eggs or insects that emerged during the treatment.

*Molecular docking study*

Molecular docking was employed to theoretically assess carvacrol's potential antibacterial, antifungal, and insecticidal activities.

*Ligand preparation*

Carvacrol was downloaded from the PubChem database in SDF format under PubChem CID: 10364. The LigPrep module from Schrödinger software (version 11.5) was used to prepare this molecule using the OPLS3 force field. Ionization state optimization of the molecule was done at pH 7.0 ± 2.0, generating up to 32 possible stereoisomers.[16,17]

*Protein preparation*

The proteins used for docking, including *Escherichia coli* beta-ketoacyl-[acyl carrier protein] synthase (PDB ID: 1FJ4), *Staphylococcus aureus* nucleoside diphosphate kinase (PDB ID: 3Q8U), *Aspergillus niger* beta-1,4-endoglucanase (PDB ID: 5I77), acetylcholinesterase (PDB ID: 6ARY), and Chitin Synthase 2 (PDB ID: 7STM) were retrieved from the Protein Data Bank. The protein preparation process involved refining the structures by adding hydrogen atoms, correcting bond orders, removing water molecules, assigning hydrogen bonds, optimizing receptor atom charges, and minimizing energy using the OPLS3 force field.[18,19]

*Glide standard precision (SP) ligand docking*

Flexible ligand docking was performed using the SP (Standard Precision) mode in Glide from Schrödinger-Maestro version 11.5. Non-cis/trans amide bonds were penalized during the docking process. Van der Waals interactions were scaled for the ligand atoms with a factor of 0.80, and the partial charge cutoff was set to 0.15. The docking results were evaluated based on the glide score derived from the ligands' energy-minimized poses. The pose with the lowest glide score for each ligand was selected as the best docking result.[20,21]

*ADME/toxicity predictions*

ADME/toxicity predictions were conducted by evaluating absorption, distribution, metabolism, and excretion parameters using the QikProp module in Maestro version 11.5 of the Schrödinger suite. This analysis considered various physicochemical and pharmacokinetic properties, such as molecular weight, hydrogen bond donors and acceptors, total solvent-accessible surface area, blood-brain barrier partition coefficient, octanol/water partition coefficient, and aqueous solubility, to validate the hypothesis. Furthermore, the ProTox-II platform assessed the organic toxicities and toxicological attributes, including $LD_{50}$.[22]

*Statistical analysis*

Statistical analysis was conducted using SPSS for Windows® (version 21) statistical software program. To ensure the study's validity, the t-test and Shapiro-Wilks tests were employed to assess homogeneity and normality. The differences between the mean values of the groups was evaluated using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test. Statistical significant difference was set at P-value < 0.05. The probit technique was also employed to determine the $LC_{50}$ and $LC_{95}$ fatal concentrations, along with their associated confidence intervals.

## Results and Discussion

*Antimicrobial activity of carvacrol*

The MIC, MBC, and MFC values of Carvacrol against the test organisms are presented in Tables 1 and 2. The MIC values were 4.125% against Escherichia coli and Staphylococcus aureus and 2.06% against *Listeria innocua* and *Pseudomonas aeruginosa*. Whereas the MBC values were 16.5% against E. coli and S. aureus and 8.75%

against *L. innocua* and *P. aeruginosa*. For fungal strains, carvacrol demonstrated highly effective inhibitory activity (Table 2). The minimum inhibitory concentration (MIC) values were 2.06% against *Aspergillus niger and Penicillium* digitatum and 4.125% against *Candida glabrata*. However, the minimum fungicidal concentration (MFC) values were above 16%, indicating moderate fungicidal activity.

**Table 1:** Antibacterial activity of carvacrol

| | *Esxherichia coli* | *Staphylococcus aureus* | *Listeria innocua* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|
| **MIC (% w/w)** | 4.125 | 4.125 | 2.060 | 2.060 |
| **MBC (% w/w)** | 16.50 | 16.50 | 8.75 | 8.75 |

MIC and MFC values exceeding 16% reflect limited effectiveness in completely eradicating the fungi studied. Carvacrol is a primary bioactive compound found in the essential oils of various aromatic and medicinal plants, particularly Origanum and Thymus species. While the antimicrobial properties of essential oils have been extensively studied, research on the antimicrobial potential of isolated bioactive molecules from these oils remains limited. This study further contributes to the body of work assessing the antimicrobial activity of natural plant-derived molecules. In this regard, several researchers have investigated the antimicrobial activity of monoterpenes, a class of phytochemicals to which carvacrol belong. The antimicrobial activity of carvacrol stems from its ability to disrupt the cellular membrane of microorganisms, leading to altered membrane permeability and leakage of essential cellular components, such as ions and proteins.[23] This disruption is linked to the functional groups present, with the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons playing crucial roles in its antimicrobial action.[24,25] This antimicrobial action of carvacrol often results in cell death, particularly in Gram-negative and Gram-positive bacteria. Numerous studies have shown that carvacrol is effective against many pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[26] Additionally, carvacrol has demonstrated antifungal activity, notably against Candida albicans.[26] The effectiveness of carvacrol can vary based on concentration, the type of microorganism targeted, and environmental conditions. Its potential as a natural antimicrobial agent makes it a promising candidate for applications in the food industry, medicine, and the preservation of biological products. However, further research is required to understand the mechanisms of carvacrol's antimicrobial action and its long-term impact on antimicrobial resistance.

**Table 2:** Antifungal activity of carvacrol

| | *Aspergillus niger* | *Candida glabrata* | *Penicillium digitatum* |
|---|---|---|---|
| **MIC (% w/w)** | 2.060 | 4.1250 | 2.060 |
| **MFC (% w/w)** | 33 | >33 | 33 |

*Insecticidal activity of carvacrol*
*Toxicity against adults*

This study evaluated various concentrations of carvacrol (0, 1, 5, 10, and 20 µL/L of air) for their fumigation toxicity against adult *Callosobruchus maculatus*. The goal was to assess the efficacy of each concentration on insect mortality. Adult mortality was monitored every 12 hours over 48 hours. The results demonstrate a clear concentration-response relationship, with increasing carvacrol concentration leading to higher insect mortality (Figure 1). From the results as shown in Figure 1, carvacrol demonstrated a significant insecticidal effect on the longevity of *Callosobruchus maculatus* adults. Insect mortality increased with both the concentration and the duration of exposure to carvacrol. A total mortality rate (100%) was observed at a

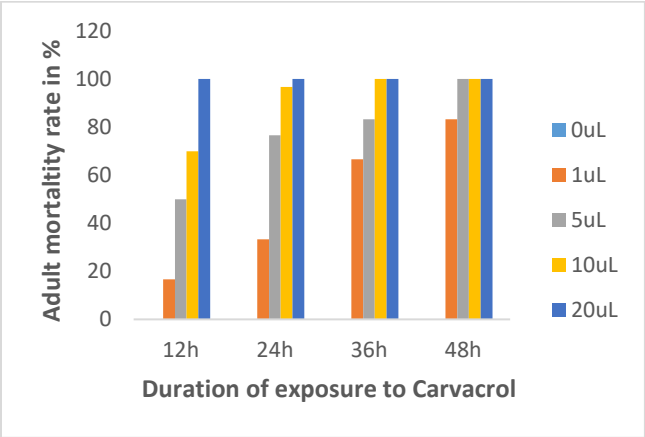


**Figure 1:** Insecticidal activity of carvacrol against *Calosobruchus maculatus* adults

concentration of 20 µL/L of air after 12 hours of exposure, highlighting the potent insecticidal efficacy of this essential oil component. Even at lower concentrations, the effect remained substantial: the lowest concentration tested (1 µL/L of air) induced 83.33% mortality after 48 hours of exposure, though this same concentration only caused 16.67% mortality after 12 hours.

*Toxicity against female fertility*
The effect of carvacrol on egg-laying was systematically studied, with results shown in Figure 2. The result revealed a clear inverse correlation between the concentration of the carvacrol and the number of eggs laid. At the lowest concentration (1 µL/L), the average number of eggs per female was 76, representing a significant reduction in fertility of 67.7% compared to the control group. No egg-laying was observed at the highest concentration (20 µL/L), indicating a complete 100% suppression of egg production. In contrast, in the control group, females of *C. maculatus* laid an average of 179.67 eggs per female.

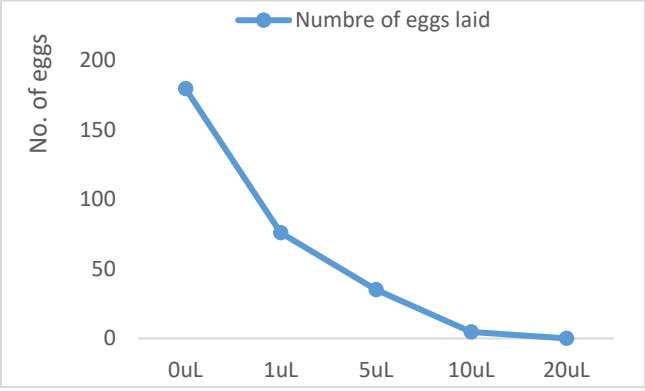


**Figure 2:** Effect of carvacrol against egg-laying in *Callosobruchus maculatus* females

*Effect on adult emergence*
From the results as presented in Figure 3, carvacrol treatments significantly reduced the number of adult emergence compared to the control groups, where the emergence rate was highest (102.67 individuals). At the lowest dose tested (1 µL/L), approximately 35 individuals completed their life cycle and emerged. However, this

number rapidly decreased as the carvacrol concentration increased, indicating a concentration-dependent effect. At the highest concentration, carvacrol completely inhibited adult emergence, demonstrating its toxic effect against *C. maculatus*. This drastic reduction in the number of insects that completed their development highlights the impact of carvacrol on the entire life cycle of the insect pest, suggesting that this compound could be used as an effective biological control method to prevent the spread of this harmful species.

The results of this study suggest that carvacrol is a promising natural insecticide for controlling insect pests, and several studies have confirmed its effectiveness. For instance, it has been reported that essential oils rich in carvacrol, such as Origanum compactum oil, exhibit significant insecticidal activity against *Callosobruchus maculatus*.[27] Similarly, [28] showed that the $LC_{50}$ of carvacrol, obtained through leaf-dipping bioassay against *P. shantungensis* nymphs, was 56.74 mg/L, indicating high toxicity against this insect.

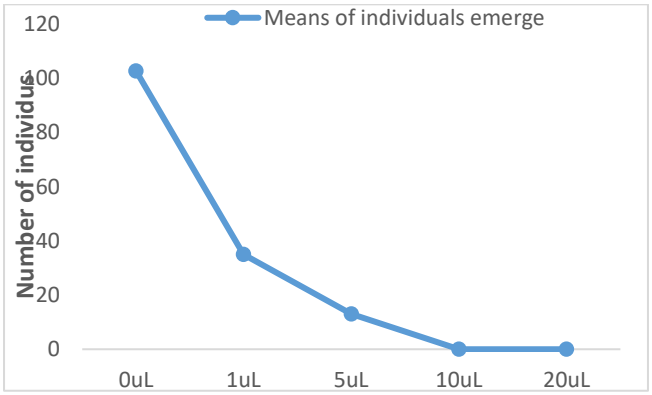


**Figure 3:** Effect of carvacrol against adult emergence in *Callosobruchus maculatus*

More recently, it has been demonstrated that carvacrol inhibits growth and induces mortality in *Spodoptera frugiperda* larvae while affecting developmental parameters and enzymatic activities related to digestion and detoxification.[29] These findings are consistent with the observations from the present study, indicating that carvacrol has a strong potential to control *Callosobruchus maculatus* populations by acting on adults, eggs, and larvae. Its multifaceted action, combined with its plant-based origin, makes it a viable option for effective biological control of harmful insects, thereby reducing dependence on traditional chemical insecticides.

*In silico molecular docking data*

In the in silico antimicrobial activity study, carvacrol showed higheractivity against E. coli and S. aureus with a glide gscore of -6.516 and 4.905 kcal/mol, glide emodel of -35.408 and -33.782 kcal/mol, and glide energy of -25.741 and -24.001 kcal/mol, respectively (Table 3).

With respect to the antifungal activity, carvacrol showed remarkable inhibitory activity against Aspergillus niger with a glide gscore of -5.321 kcal/mol, a glide emodel of -27.771 kcal/mol, and a glide energy of -21.205 kcal/mol (Table 3). For the insecticidal activity, carvacrol exhibited remarkable inhibitory activity against acetylcholinesterase and chitin synthase, with a glide score of -6.747 and -5.442 kcal/mol, respectively, a glide emodel of -35.464 and -36.678 kcal/mol, and a glide energy of -25.354 and -25.386 kcal/mol (Table 3).

2D and 3D interaction analysis of carvacrol with different active sites showed the carvacrol has established one hydrogen bond with THR 302 residue in the active site of Escherichia coli beta-ketoacyl-[acyl carrier protein] synthase (Figures 4A and 5A). Moreover, in the active site of *Aspergillus niger* beta-1,4-endoglucanase, carvacrol forms a single Pi-cation bond with residue ARG 102. at the same time, carvacrol established two hydrogen bonds with residues SER 196 and TRP 201 and one Pi-Pi stacking bond with residue TRP 197 in the active site of *Aspergillus niger* (Figures 4C and 5C). Furthermore, in the insecticidal activity, carvacrol established one hydrogen bond with residue ILE 321 in the active site of acetylcholinesterase (Figures 4D and 5D) and one hydrogen bond with residue GLU 321 in the active site of chitin synthase 2 (Figures 4E and 5E).

*Predicted ADME/toxicity of carvacrol*

The bioavailability of an active compound is influenced by its absorption, distribution, metabolism, and excretion (ADME) properties,

**Table 3:** Docking results of carvacrol with different receptors

| | | Glide gscore (kcal/mol) | Glide emodel (kcal/mol) | Glide energy (kcal/mol) |
|---|---|---|---|---|
| Antibacterial activity | *Escherichia coli* (PDB ID: 1FJ4) | -6.516 | -35.408 | -25.741 |
| | *Staphylococcus aureus* (PDB ID: 3Q8U) | -4.905 | -33.782 | -24.001 |
| Antifungal activity | *Aspergillus niger* (PDB ID:5I77) | -5.321 | -27.771 | -21.205 |
| Insecticidal activity | acetylcholinesterase (PDB ID: 6ARY) | -6.747 | -35.464 | -25.354 |
| | Chitin Synthase 2 (PDB ID: 7STM) | -5.442 | -36.678 | -25.386 |

which are closely related to its physicochemical characteristics. Carvacrol has a molecular weight of less than 500 g/mol. The number of hydrogen bond donors and acceptors was within acceptable limits (≤5 and ≤10, respectively) (Table 4). Oral bioavailability is influenced by the solvent-accessible surface area, with carvacrol having acceptable values between 300 and 1000. The blood-brain partition coefficient, which reflects the potential of a compound to cross the blood-brain barrier, was within the acceptable range of −3 to 1.2. The predicted oral absorption rate was 100% (Table 4).

**Table 4:** ADME prediction of carvacrol

| Parameter | Carvacrol |
|---|---|
| Molecular mass (acceptable range: 500 g/mol) | 150.22 |
| Total solvent accessible surface area using a probe with a 1.4 radius (acceptable range: 300–1000 radius). | 394.212 |
| Hydrogen bond donor (acceptable range: ≤5) | 1 |
| Hydrogen bond acceptor (acceptable range: ≤10) | 0.75 |
| Predicted octanol/water partition coefficient (acceptable range: −2–6.5) | 3.298 |
| Predicted aqueous solubility (S) in mol/dm$^{-3}$ (acceptable range: −6.5–0.5) | -2.33 |
| Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells is a model for the gut-blood barrier (<25-poor, >500-great) | 3683.827 |
| Predicted blood-brain partition coefficient (acceptable range: -3 to 1.2) | 0.071 |
| QPlogKp | -1.816 |
| Predicted human oral absorption on 0%–100% scale (<25% is poor, and >80% is high) | 100 |

Toxicity studies are crucial in evaluating the potential risks substances or products may pose to living organisms, including humans, animals, and the environment. These assessments ensure the safety of various chemicals, pharmaceuticals, pesticides, food additives, and consumer

goods before widespread use. Toxicity studies help mitigate risks and guide regulatory decisions by identifying harmful effects early. Our research focused on predicting the toxicological profile of carvacrol, a bioactive compound commonly found in essential oils. The in-silico toxicity predictions indicated that carvacrol may pose several toxicological risks, including neurotoxicity and respiratory toxicity, which could impact both the central nervous system and respiratory health. Additionally, carvacrol was flagged for its potential to cross the

**Table 5:** Toxicity prediction for carvacrol

| Target | Prediction | Probability |
|---|---|---|
| Hepatotoxicity | Inactive | 0.75 |
| Neurotoxicity | Active | 0.52 |
| Nephrotoxicity | Inactive | 0.72 |
| Respiratory toxicity | Active | 0.64 |
| Cardiotoxicity | Inactive | 0.99 |
| Carcinogenicity | Inactive | 0.60 |
| Immunotoxicity | Inactive | 0.96 |
| Mutagenicity | Inactive | 0.99 |
| Cytotoxicity | Inactive | 0.89 |
| BB-barrier | Active | 0.93 |
| Ecotoxicity | Active | 0.54 |
| Clinical toxicity | Inactive | 0.65 |
| Nutritional toxicity | Inactive | 0.93 |
| Aryl hydrocarbon Receptor (AhR) | Inactive | 1 |
| Androgen Receptor (AR) | Inactive | 1 |
| Androgen Receptor Ligand Binding Domain (AR-LBD) | Inactive | 1 |
| Aromatase | Inactive | 1 |
| Estrogen Receptor Alpha (ER) | Inactive | 1 |
| Estrogen Receptor Ligand Binding Domain (ER-LBD) | Inactive | 1 |
| Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | Inactive | 1 |
| Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | Inactive | 1 |
| Heat shock factor response element (HSE) | Inactive | 1 |
| Mitochondrial Membrane Potential (MMP) | Active | 1 |
| Phosphoprotein (Tumor Suppressor) p53 | Inactive | 1 |
| ATPase family AAA domain-containing protein 5 (ATAD5) | Inactive | 1 |
| Thyroid hormone receptor alpha (THRα) | Inactive | 0.90 |
| Thyroid hormone receptor beta (THRβ) | Inactive | 0.78 |
| Transtyretrin (TTR) | Inactive | 0.97 |
| Ryanodine receptor (RYR) | Inactive | 0.98 |
| GABA receptor (GABAR) | Inactive | 0.96 |
| Glutamate N-methyl-D-aspartate receptor (NMDAR) | Inactive | 0.92 |
| alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR) | Inactive | 0.97 |
| Kainate receptor (KAR) | Inactive | 0.99 |
| Acetylcholinesterase (AChE) | Inactive | 0.75 |
| Constitutive androstane receptor (CAR) | Inactive | 0.98 |
| Pregnane X receptor (PXR) | Inactive | 0.92 |
| NADH-quinone oxidoreductase (NADHOX) | Inactive | 0.97 |
| Voltage gated sodium channel (VGSC) | Inactive | 0.95 |
| Na+/I- symporter (NIS) | Inactive | 0.98 |

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| | | |
|---|---|---|
| Cytochrome CYP1A2 | Inactive | 0.80 |
| Cytochrome CYP2C19 | Inactive | 0.50 |
| Cytochrome CYP2C9 | Active | 0.70 |
| Cytochrome CYP2D6 | Inactive | 0.88 |
| Cytochrome CYP3A4 | Inactive | 0.97 |
| Cytochrome CYP2E1 | Inactive | 1 |

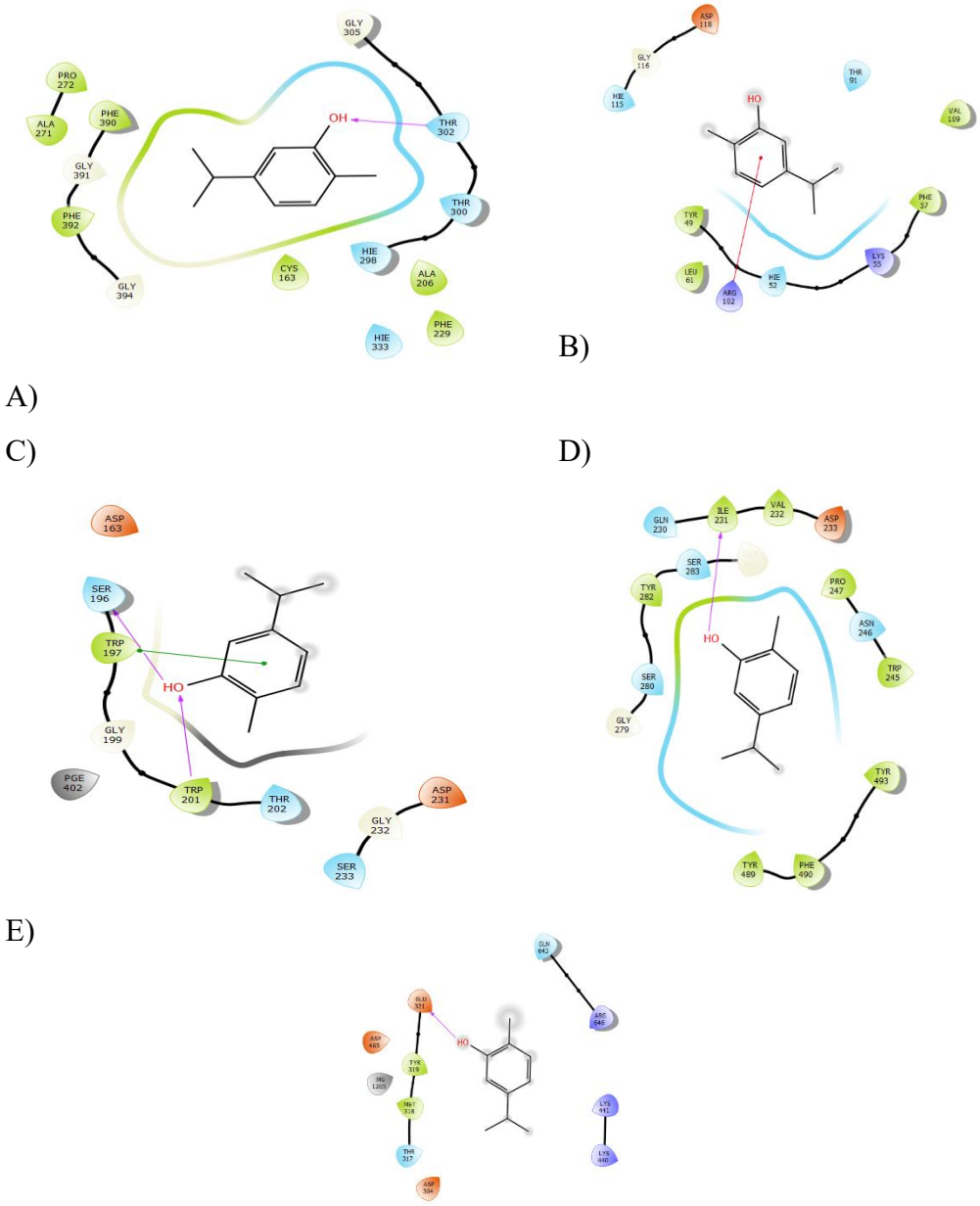


**Figure 4:** The 2D visualization of carvacrol interactions with the protein active sites. (A): beta-ketoacyl-[acyl carrier protein] synthase from *Escherichia coli*. (B): *Staphylococcus aureus* nucleoside diphosphate kinase. (C): beta-1,4-endoglucanase from *Aspergillus niger*. (D): acetylcholinesterase. (E): chitin synthase 2

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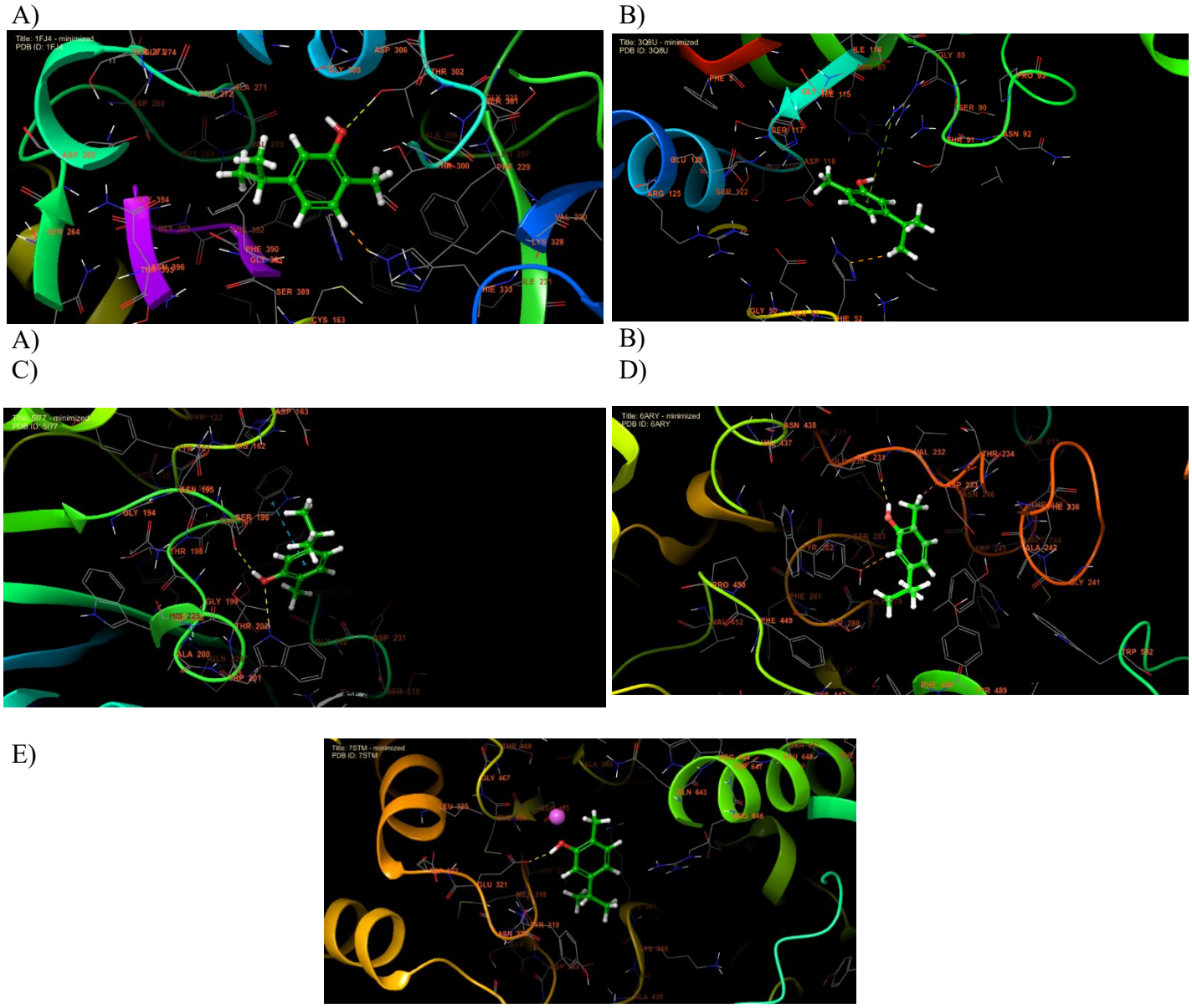


**Figure 5:** The 3D visualization of carvacrol interactions with the protein active sites. (A): beta-ketoacyl-[acyl carrier protein] synthase from *Escherichia coli*. (B): *Staphylococcus aureus* nucleoside diphosphate kinase. (C): beta-1,4-endoglucanase from *Aspergillus niger*. (D): acetylcholinesterase. (E): chitin synthase 2.

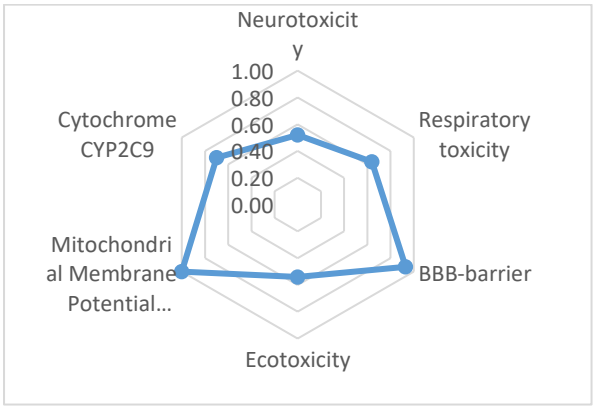


Figure 6: Toxicological profile of carvacrol in human, animal, and environmental health

blood-brain barrier (BBB), which raises concerns about its direct effects on the brain. Environmental toxicity was also predicted, suggesting that carvacrol might negatively affect aquatic and terrestrial ecosystems. Furthermore, carvacrol was found to influence the mitochondrial membrane potential (MMP), which is critical for cellular energy production and overall cell function. Notably, carvacrol showed potential interactions with the cytochrome P450 enzyme CYP2C9, which is involved in drug metabolism and could lead to adverse drug interactions (Table 5 and Figure 6). These findings highlight the need for comprehensive experimental validation to confirm the predicted toxic effects and guide safe usage in various applications.

## Conclusion

This study demonstrated the significant efficacy of carvacrol as an antimicrobial and insecticidal agent. Its antibacterial, antifungal, and insecticidal properties and mode of action highlight its potential for biological and industrial applications. The *in vitro* results show that

carvacrol exhibits notable inhibitory effects on the test bacterial and fungal strains. At the same time, its insecticidal activity proved particularly effective against *Callosobruchus maculatus,* with complete inhibition of adult emergence and egg-laying. The *in silico* analysis also confirmed these findings by revealing relevant molecular interactions with specific targets in bacteria, fungi, and insects. These results suggest that carvacrol could represent a promising natural alternative to synthetic pesticides and antimicrobial agents, contributing to sustainable pest management and reducing the environmental and health risks associated with traditional chemical products. However, further studies are needed to assess the long-term effects of carvacrol, particularly as it concern its potential toxicity and impact on antimicrobial resistance.

## Conflict of interest

The author reports no conflicts of interest in this work.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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