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MECHANISTIC INSIGHT ANTIMICROBIAL POTENTIAL OF CLOVE OIL: GRID BASED DOCKING APPROACH

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ABSTRACT: Objective: The prevalence of antibiotic resistance in bacteria is currently seeing a significant increase, which presents a genuine danger to global health. Of particular concern are contaminations produced by methicillin-resistant Penicillin-tolerant Methicillin-resistant Staphylococcus aureus (MRSA) A vancomycin-resistant strain of Streptococcus pneumoniae, Enterococcus, and Mycobacterium tuberculosis has emerged due to the resistance developed by major populations of these organisms against several recognized antimicrobials. This condition is driving the quest for new antibacterial medications that can block crucial bacterial targets and are not influenced by present mechanisms of resistance to chemotherapeutic agents. Recent research has been dedicated to studying the aminoacyl-tRNA synthetase (AaRS) enzymes in order to produce antibacterial medications in this field. This project focuses on the identification of eugenol and Caryophyllene, which are natural plant volatile oils, as ligands. The inhibitory activity of these ligands against the aminoacyl-tRNA synthetase (AaRS) and DHFR enzyme has been evaluated using in-silico techniques, namely the docking technique.

Methods: The present study employed a molecular docking technique to attempt the identification of inhibitors for aminoacyl-tRNA synthetase (AaRS) enzyme and DHFR. The binding between molecules has been determined using the Auto Dock software, which employs a grid-based docking technique. The 2D structures of the compounds were built, converted to 3D, and then energetically minimized using the Merck Molecular Force Field (MMFF) until reaching a rms gradient of 0.01.

Results: The molecular docking result revealed that eugenol and Caryophyllene showed encouraging docking score. The docking score found to be -5.19 & -6. 49 against IIes protein whereas -6.1 &-6.7 kcal mol^{-1} respectively against DHFR enzyme.

Conclusion: The interaction of ligand hits to targeted site and docking score finding it can be predicted that volatile oil present in clove oil exhibited good inhibitor of DHFR enzyme.

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Keywords: Eugenol and Caryophyllene, IIes, DHFR synthase and *in-silico* molecular docking.

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1.INTRODUCTION

Molecular docking is a commonly used method in structure-based drug design (SBDD) because it can accurately anticipate the arrangement of small-molecule ligands in the target binding site. Molecular docking emerged as an essential method in drug discovery during the 1980s following the development of the initial algorithms. In addition, molecular docking methods offer precise quantitative assessments of the energy involved in binding, resulting in rankings of docked molecules based on the strengths of the interactions between ligands and receptors. The execution of these activities involves the utilization of molecular docking programs in a repetitive fashion, where the conformation of the ligand is evaluated utilizing specific scoring systems. Iteratively, this technique is performed until it reaches a minimal energy solution [1-4]. The current surge in the incidence of antibiotic resistance among microorganisms is a genuine menace to global health. More precisely, contaminations produced by methicillin-resistant Penicillin-tolerant Methicillin-resistant Staphylococcus aureus (MRSA) Staphylococcus aureus Vancomycin is effective against Streptococcus pneumoniae. Due to their resistance to multiple recognized antimicrobials, Enterococcus and Mycobacterium tuberculosis are the two most prevalent organisms. This situation is inspiring researchers to seek out novel antibacterial medications that might hinder essential bacterial targets and remain unaffected by the mechanisms of resistance to currently employed chemotherapeutic agents. Presently, research efforts in the field of antibacterial medication development have primarily concentrated on the aminoacyl-tRNA synthetase (AaRS) enzymes. These enzymes play essential roles in protein biosynthesis by facilitating the formation of aminoacyl-tRNAs. In this study, the ligand properties of eugenol and caryophyllene, which are natural volatile oils found in clove plants, were investigated. The inhibitory activity of these oils on aminoacyl-tRNA synthetase (AaRS) and DHFR enzymes was analyzed using an *in-silico* docking approach. [5-8]

2. MATERIALS AND METHODS

Molecular docking studies

Ligand Preparation:

The 2D structures of eugenol and caryophyllene were created using ChemSketch [9]. These 2D

structures were then translated into their 3D structures by optimizing their 3D geometry. The optimized structure was saved in PDB format to ensure compatibility with AutoDock. The provided ligand was characterized by its fundamental structures as follows:

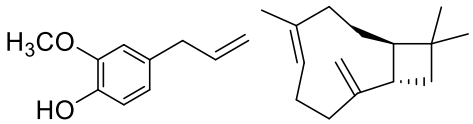


Figure 1: 2D structure of eugenol and caryophyllene.

Preparation of the grid file

Autodock defined the regions of interest by creating a grid box around the active sites and examining the grid area. The grid box is essential in the docking process as it encompasses all the amino acids in the active sites required for binding, except those found in the receptor. The grid box contains three thumbwheel widgets that allow for the adjustment of the number of points in the x, y, and z dimensions. The table 1 [10-12] provides the spacing and grid points for the IleS receptor of S. aureus and DHFR of C. albicans in the current investigation.

Table 1. Grid parameters used in current docking analysis

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	IleS	60	60	60	1.000	27.914	89.835	81.39
2	DHFR	40	40	40	0.758	3.334	-0.246	27.626

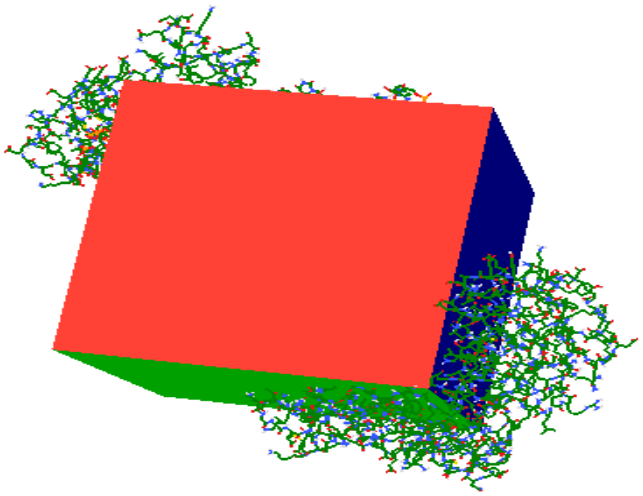


Figure 2: Grid box covering all active sites in IleS receptor.

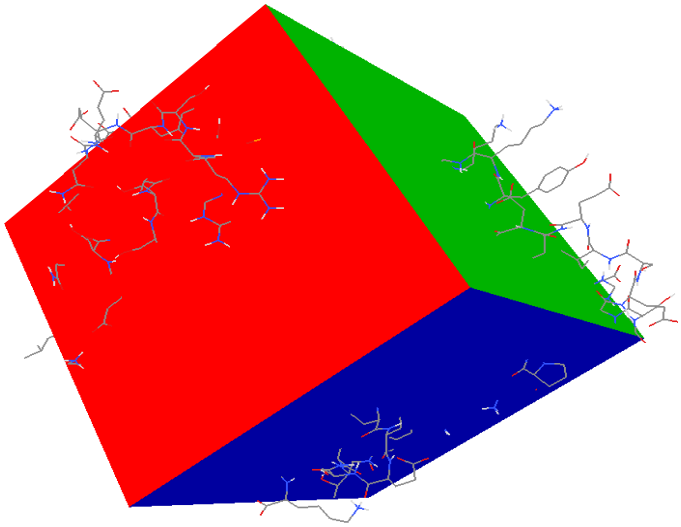


Figure 3: Grid box covering all active sites in DHFR receptor

Preparation of the docking file

The computations were performed using Autodock 4.2 as the docking tool. The docking studies were conducted using Pymol, Chimera, DS visualizer, and MMP Plus [13-16].

Docking Study

Crystal structure

The protein's crystal structure, which includes the IleS and DHFR receptor, was obtained via the Protein Data Bank portal. The Protein Data Bank [17-21] contains comprehensive information on the structure of all receptors. The intricate ligand was isolated using Chimera software for each of the target receptors.

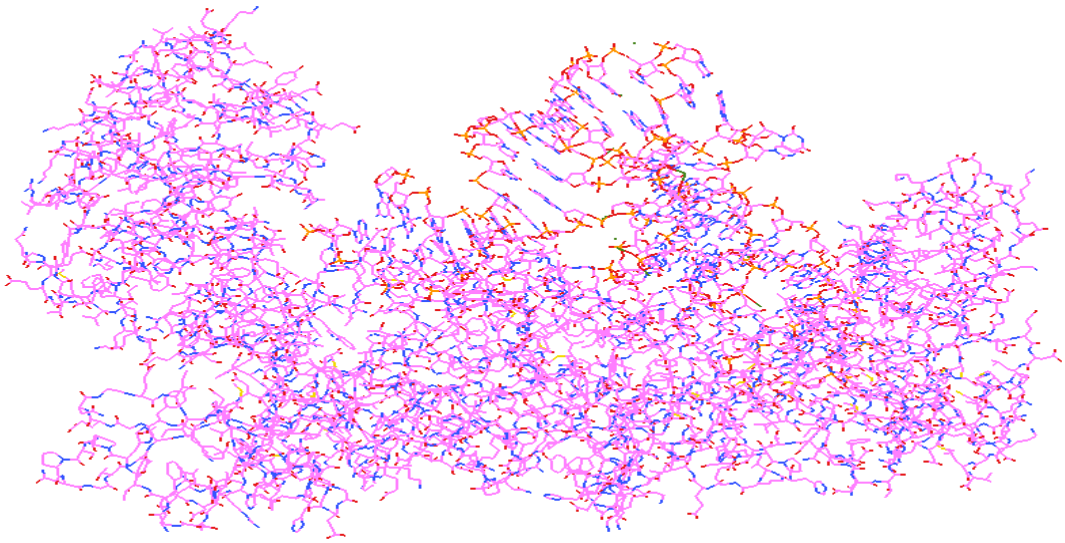


Figure 4: Crystal structure of IleS receptor (PDB ID-1ffy)

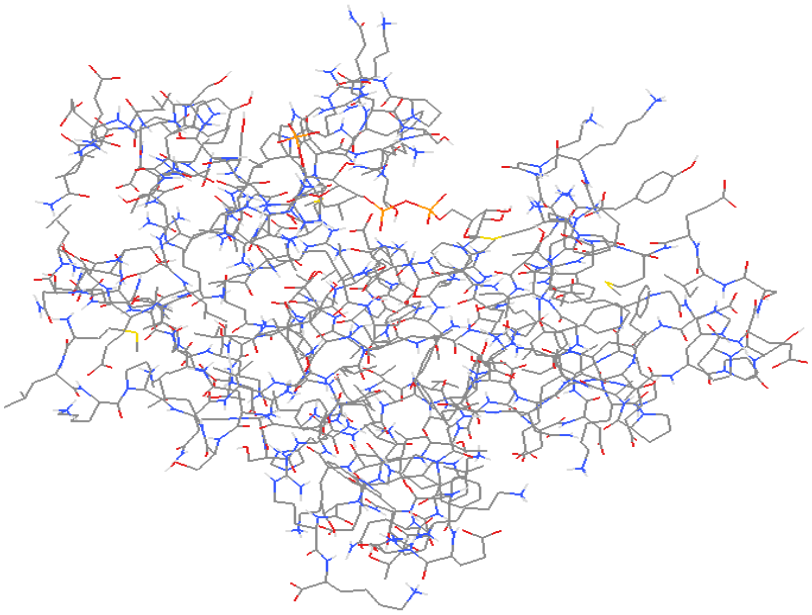


Figure 5: Crystal structure of DHFR receptor (PDB ID-4hoe)

Processing of Protein

All of the downloadable receptor proteins consist of only one chain, specifically chain A, which has been chosen for experimental purposes. The complex ligand has been extracted from this chain. The ligand that was attached to the macromolecular complex was isolated using the Chimera software [22-25].

Molecular Docking Simulation Studies

The Autodock software was used to accomplish the docking of the ligand's eugenol and caryophyllene against the IleS and DHFR receptors. The flexibility of all ligand bonds was maintained, whereas no flexibility was introduced to the receptor residues [26-29].

Toxicity & ADME-T Studies

The ligand molecules, namely eugenol and caryophyllene, were analyzed using the online tool OSIRIS to anticipate the existence of any hazardous groups and assess their ADME-T characteristics [30].

3. RESULTS AND DISCUSSION

A search was performed to discover new antibacterial substances that can hinder targets that are vital to bacteria, while remaining unaffected by resistance mechanisms to current chemotherapeutic drugs. Recent antibacterial drug development research has focused on the aminoacyl-tRNA synthetase (AaRS) enzymes. These enzymes are crucial for protein biosynthesis because they accelerate the production of aminoacyl-tRNAs (aa-rRNA). The inhibition of these enzymes' halts protein production, leading to the inhibition of bacterial growth in both laboratory and infectious settings. Throughout history, plants have been a source of optimism for discovering new therapeutic compounds due to the significant enhancement of human health brought about by herbal treatments

derived from plants. The widespread utilization of plants as remedies for various infectious diseases has prompted an ongoing search for plant chemicals with antibacterial properties. There is an urgent requirement to identify and develop new compounds to combat potentially lethal bacterial, fungal, and viral infections due to the ability of these pathogens to develop resistance to current treatment methods. These substances must possess high bioavailability, exhibit a specified level of action, and have low toxicity. Optimal compounds for this type of task are often derived from natural sources, such as plants, animals, or even microbiological organisms. Monoterpenoids has antibacterial properties, which disrupt the physiological and metabolic processes of microorganisms, inhibiting their growth and reproduction. Plant-based essential oils have long been recognized for their biological activities, including antibacterial, antioxidant, and anticancer actions. In recent years, there has been a substantial exploration of the antimicrobial characteristics of these naturally existing plant components. This research has mostly been driven by consumer apprehensions regarding the safety of synthetic food additives. The overutilization of antibiotics has led to the emergence of multidrug-resistant bacteria, which presents a significant threat to human health. In order to address this problem, it is imperative to produce potent antibacterial agents. The present study selected tannic acid, eugenol, and caryophyllene as the primary compounds for in-silico molecular docking against the target proteins DHFR and IleRS.Aminoacyl-tRNA synthetases (ARSs) are crucial components in protein synthesis and exhibit conserved enzymatic pathways across different species. Scientists have developed effective anti-infective drugs by exploiting the structural differences in the catalytic clefts of ARSs found in both infections and people, even though these structures are similar across different taxa. Advancements in genomic, proteomic, and functionomic research have uncovered other biological functions of human ARSs, going beyond their primary involvement in protein synthesis. These functions encompass the identification of unforeseen disease-related mutations, as well as changes in expression, secretion, and interactions. These studies have demonstrated that the secreted ARS proteins and their components have the potential to be valuable and untapped resources for new therapeutic targets and agents. This can be achieved through methods such as directly targeting the catalytic sites, regulating disease-associated protein-protein interactions, and creating novel biologics. Bacterial, protozoan, and other microbial disorders typically exhibit a shared heightened metabolic rate. During these occurrences, the speed at which folate is processed by the body is also increased to ensure efficient cell reproduction and the synthesis of proteins and nucleic acids. Due to the metabolic difference between folic acid antagonists and human cells, they have been used since their discovery to treat many microbial illnesses. Dihydrofolate reductase is the most extensively studied enzyme in the folate pathway due to its crucial role in maintaining the cycle. The reduction of dihydrofolate (DHF) helps to maintain a collection of various tetrahydrofolate (THF) derivatives within cells. These derivatives are then used in biosynthetic activities and reactions involving the transfer of one-carbon units. Due to its

essential role in bacterial growth, dihydrofolate reductase (DHFR) inhibitors have demonstrated potential in treating bacterial infections. The results of the in-silico molecular docking research indicate that both eugenol and caryophyllene effectively attach to the IIes protein, with binding energies of -5.19 and -6.49, respectively. Compared to caryophyllene, eugenol exhibited a more effective binding pattern with IIes, forming conventional hydrogen bonds at Met 596, with an IC 50 value of 0.12. The outcome was computed and recorded in tables 2 and 3. The binding pattern was succinctly outlined in table 4. The molecular docking analysis of specific lead compounds against the DHFR protein indicated that eugenol and caryophyllene exhibited strong interaction with DHFR, with binding energies of -6.1 and -6.7, and IC50 values of 0.10 and 0.93, respectively (as shown in table 2 and 3). The binding interaction is illustrated in Table 5 and Figure 14-17 (2D) and 10-13 (3D). The pharmacokinetic profile of the selected lead molecules indicates that they exhibit favorable pharmacokinetic characteristics. However, it is important to note that these molecules do not have any significant adverse effects such as tumorogenicity, irritating effects, or reproductive impacts, with the exception of mild mutagenicity. The figure 18-19 displays the pharmacokinetic and toxicity profiling data of the ligands. The pharmacokinetic profiles of eugenol and caryophyllene were compared in table 6-8, and it was found that both compounds demonstrated the most favorable outcomes.

Table 2: Results of docking of ligands like eugenol & caryophyllene against IleS and DHFR receptor

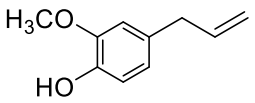
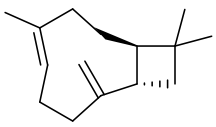
S. No	Compound	Structure	Binding Energy (kcal/mol)	
			IleS (1ffy)	DHFR (4hoe)
1	Eugenol		-5.19	-6.1
2	Caryophyllene		-6.49	-6.7

Table 3: Determination of Ki value and IC50 value

S.No.	Compound	IleS (1ffy)		DHFR (4hoe)	
		Ki	IC 50	Ki	IC 50
1	Eugenol	8.76	0.12	10.29	0.10
2	Caryophyllene	10.95	0.09	11.30	0.93

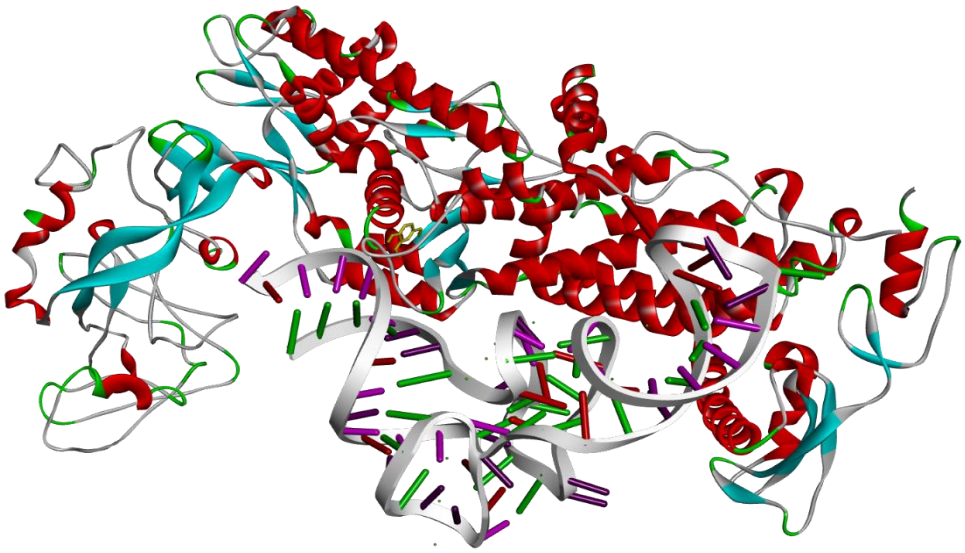


Figure 6: Binding mode of eugenol within the active site of IleS receptor.

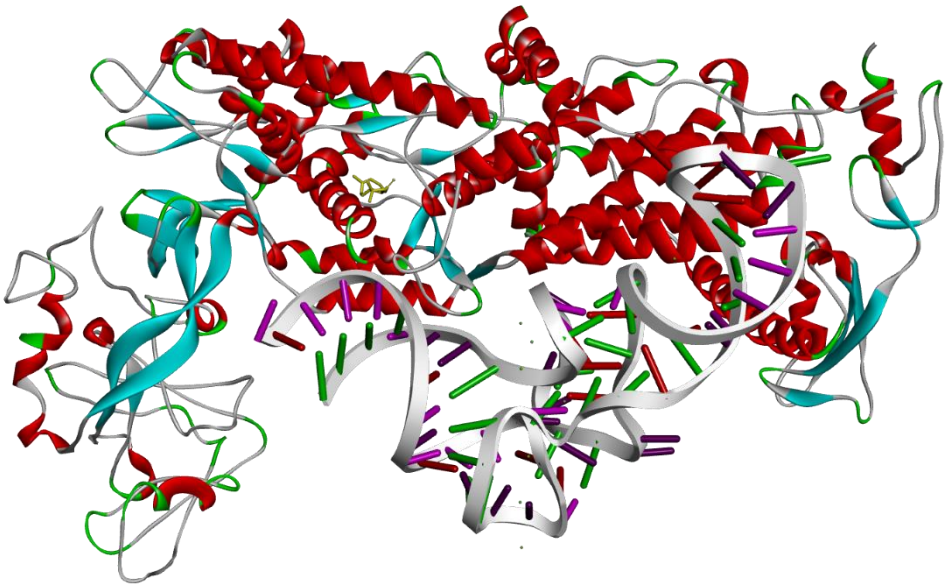


Figure 7: Binding mode of caryophyllene within the active site of IleS receptor.

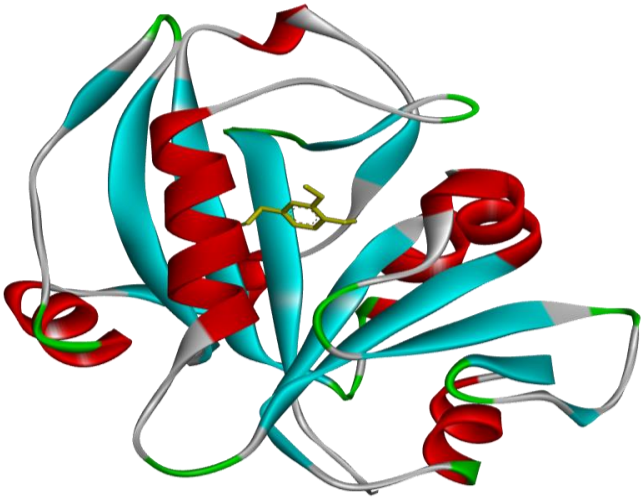


Figure 8: Binding mode of eugenol within the active site of DHFR receptor.

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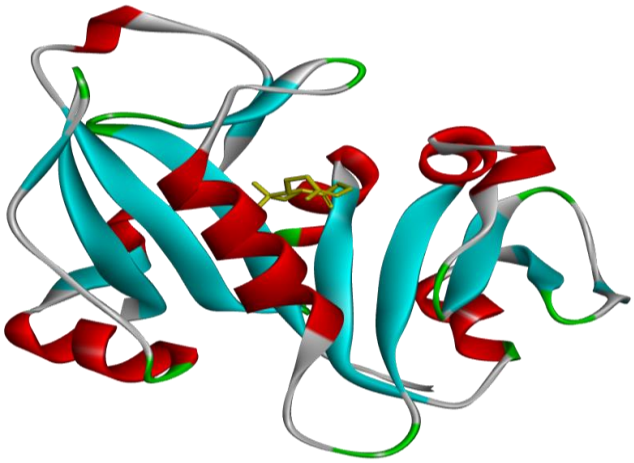

Figure 9: Binding mode of caryophyllene within the active site of DHFR receptor.

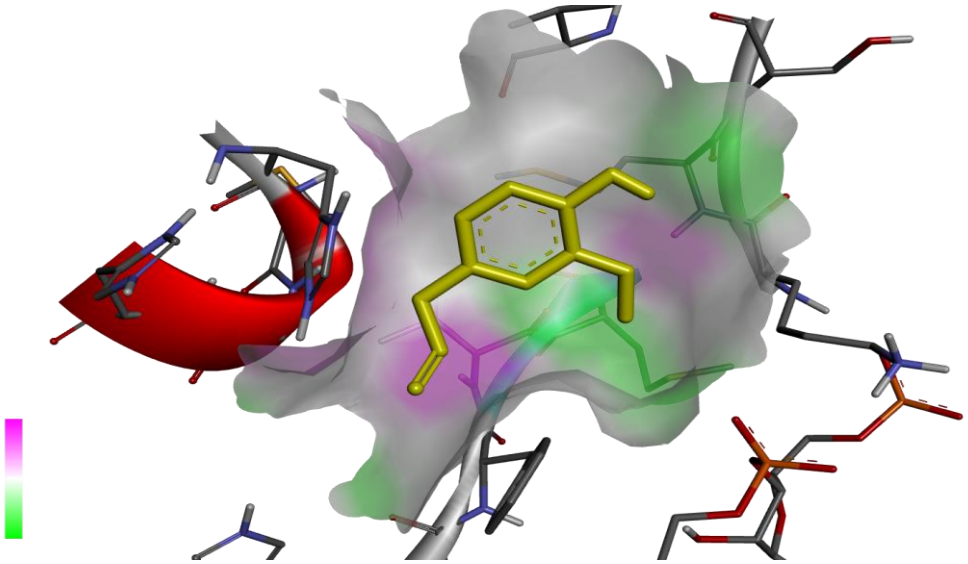

Figure 10: Three-dimensional binding mode of Eugenol within the active site of IleS receptor.

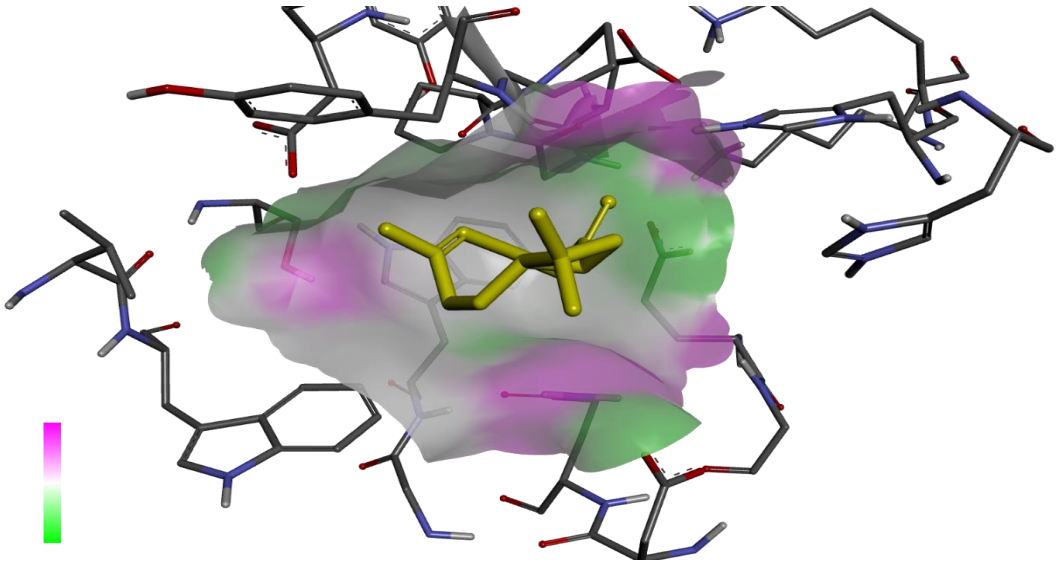

Figure 11: Three-dimensional binding mode of Caryophyllene within the active site of IleS receptor.

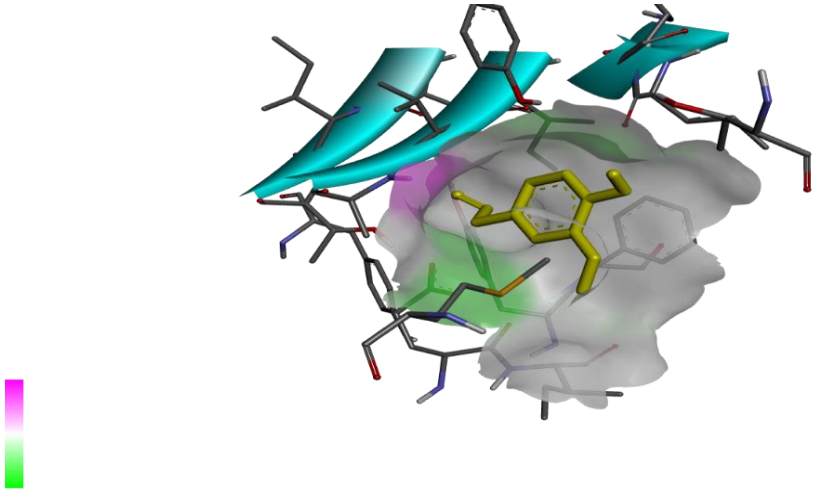

Figure 12: Three-dimensional binding mode of Eugenol within the active site of DHFR receptor.

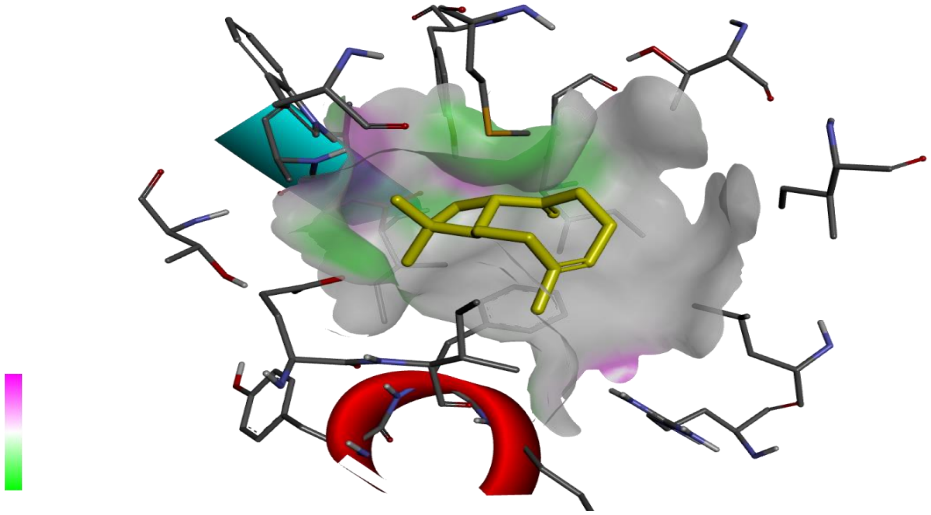

Figure 13: Three-dimensional binding mode of Caryophyllene within the active site of DHFR receptor.

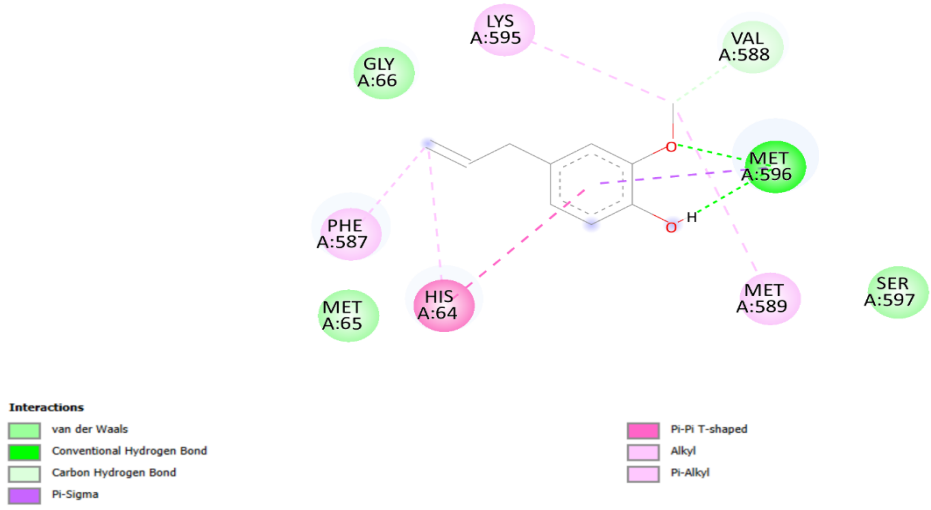

Figure 14: Two-dimensional binding mode of eugenol within the active site of IleS receptor.

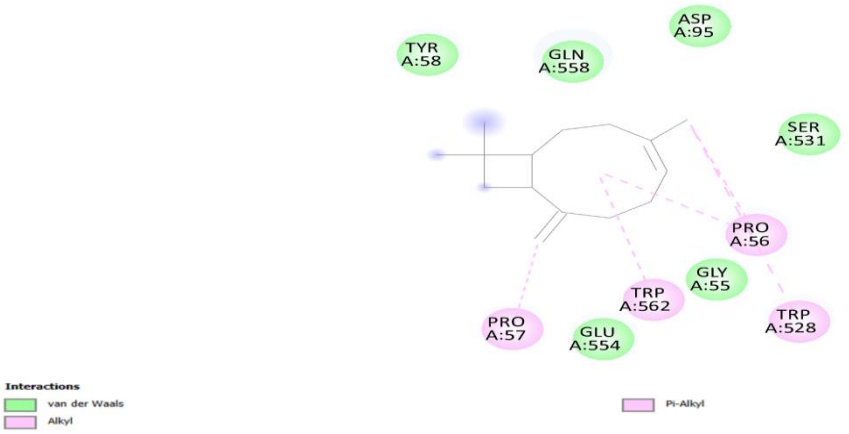

Figure 15: Two-dimensional binding mode of caryophyllene within the active site of IleS receptor.

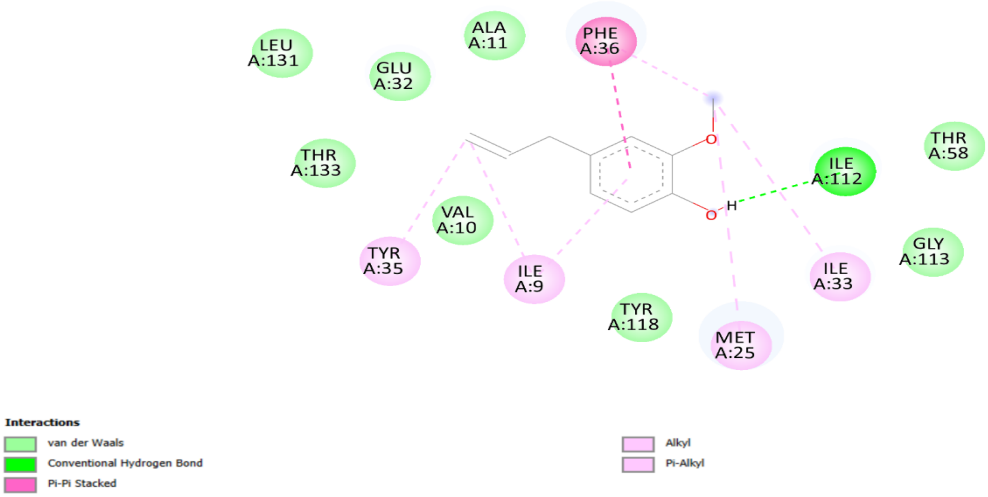

Figure 16: Two-dimensional binding mode of eugenol within the active site of DHFR receptor.

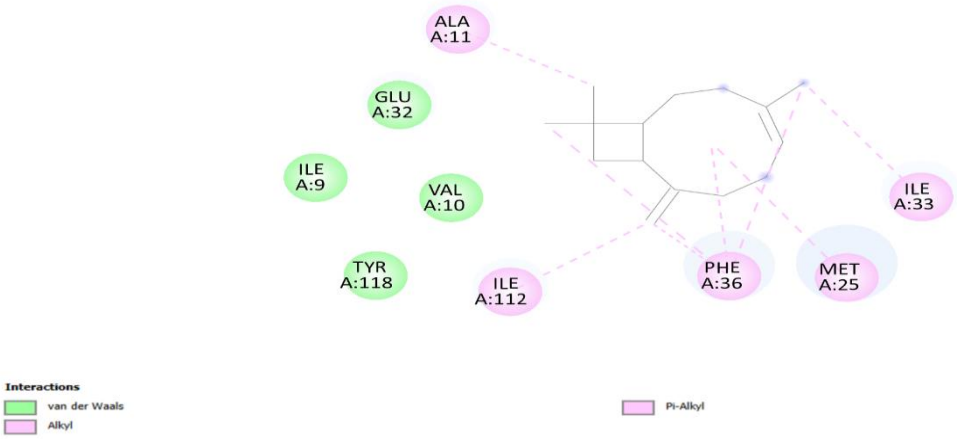

Figure 17: Two-dimensional binding mode of caryophyllene within the active site of DHFR receptor.

Table 4: Binding interaction of lead molecule with IIes protein

Compound	Conventional H bounding	Vander waals	Pi-Pi	Pi-Alkyl
Eugenol	Met 596	Val 588 Ser 597 Met 65 Gly 66	His 64	Lys 595 Phe 587 Met 589
Caryophyllene	-	Tyr 58 Gln 558 Asp 95 Ser 531 Glu 554	-	Pro 56 Trp 528 Trp 562 Pro 57

Table 5: Binding interaction of lead molecule with DHFR protein

Compound	Conventional H bounding	Vander waals	Pi-Pi	Pi-Alkyl
Eugenol	Ile 112	Thr 58 Gly 113 Tyr 118 Ala 11 Glu 32 Leu 131 Thr 133 Val 10	Phe 36	Thy 35 Ile 9 Met 25 Ile 33
Caryophyllene	-	Glu 32 Val 10 Ile 9 Tyr 118	-	Ala 11 Ile 112 Phe 36 Met 25 Ile 33

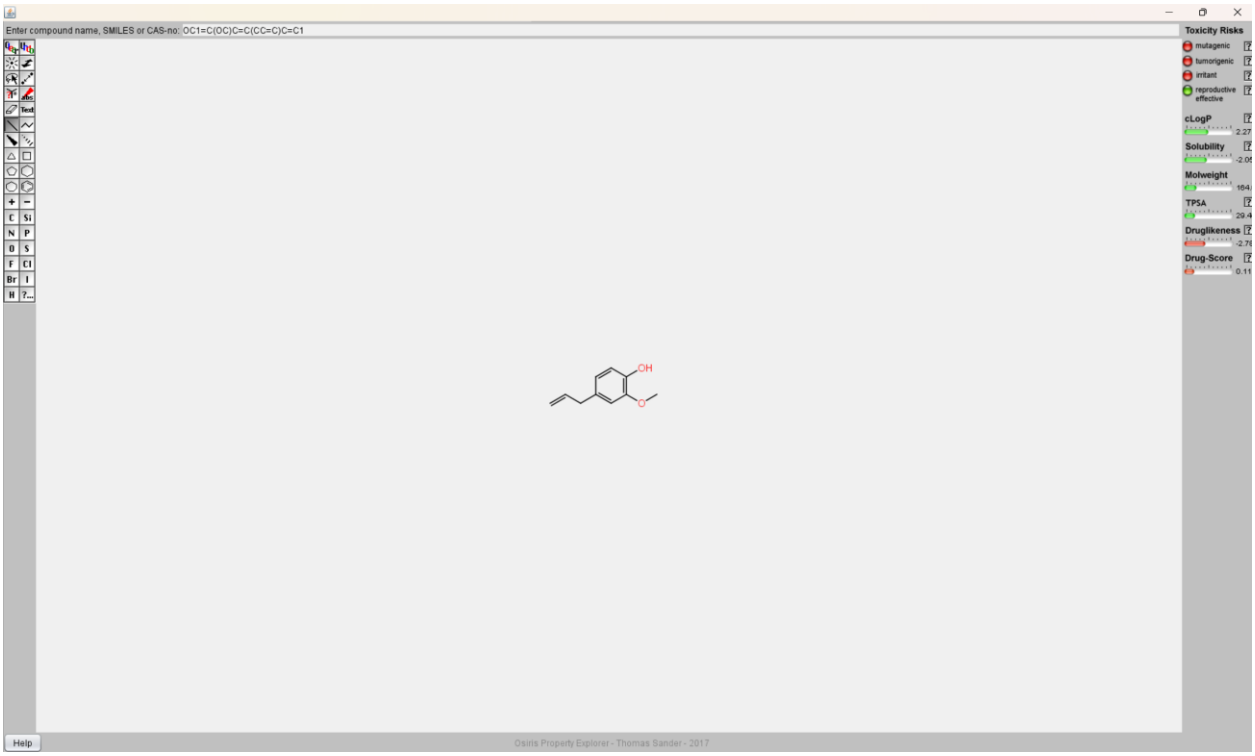

Figure 18: Pharmacokinetic and toxicity profiling of eugenol.

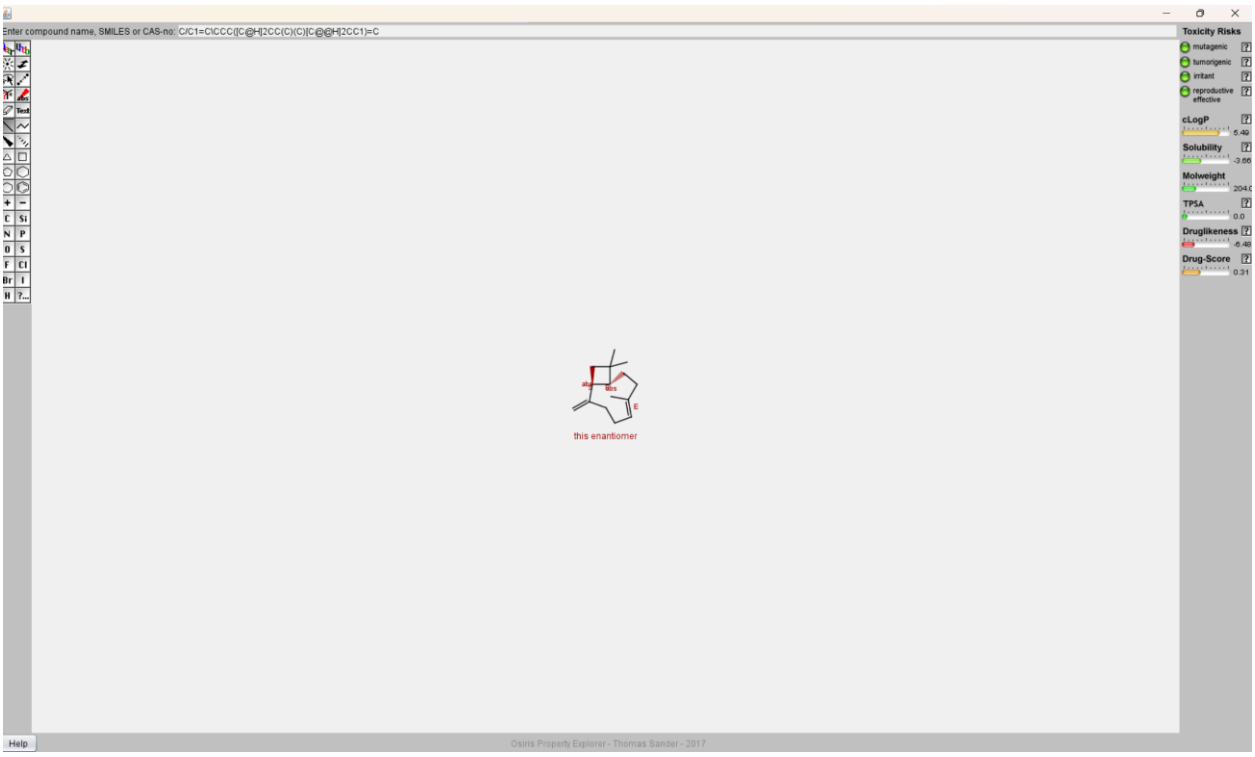

Figure 19: Pharmacokinetic and toxicity profiling of caryophyllene.

Table 6: Pharmacokinetic Profiling of lead molecules

Compound	ADMET			
	Mutagenic	**Tumorigenic**	**Irritant**	**Reproductive effectivity**
Eugenol	NO	Mild	Yes	NO
Caryophyllene	NO	NO	NO	Mild

Table 7: Lipinski Properties of lead molecules

Compound	*cLogP*	Solubility	Mol.wt.	TPSA	Drug likeness	Drug score
Eugenol	2.27	-2.6	104	20.4	2.7	0.11
Caryophyllene	5.40	-3.0	204	2.1	0.2	0.31

Table 8: Drug likeness of lead molecules

Compound	Lipinski rule of five	H bond donar (<5)	H bond acceptor (<10)
Eugenol	Yes	1	4
Caryophyllene	Yes	0	1

4. CONCLUSION

Tyrosyl-tRNA synthetase is a highly sought-after enzyme in the quest for new antimicrobial medications. The enzyme belongs to the family of aminoacyl-tRNA synthetases (aaRSs) and facilitates the formation of covalent bonds between amino acids and their corresponding tRNA molecules, resulting in the production of charged tRNA. Since aaRSs play a crucial role in protein production, its inhibition directly affects cell development. However, it is important to note that dihydrofolate reductase (DHFR) is an essential enzyme for bacterial growth. Therefore, inhibitors of IIes and DHFR have demonstrated efficacy in the treatment of bacterial infections. Essential oils show potential as novel sources of antibacterial compounds, specifically targeting bacterial infections. Oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savory, and fennel are all examples of plants and spices that have both edible and therapeutic properties, making them herbal in nature. These can be used separately or in combination with other preservation methods. While plant-derived essential oils have been used intuitively for centuries, their antibacterial effects have only recently been the focus of scientific research. The present study employed DHFR and IIes proteins to assess the antibacterial efficacy of eugenol and caryophyllene by in-silico molecular docking analysis. The findings indicated that caryophyllene exhibited a binding preference for the DHFR protein, while eugenol shown a higher affinity for both IIes and

DHFR proteins. The selected lead compounds exhibited antibacterial activity through the presence of volatile oil.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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