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Original article

Computational Docking and Virtual Screening of *Thymus vulgaris* as Potential Inhibitors for Multi-Drug-Resistant Tuberculosis (MDR-TB) Target Proteins

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Abstract

MDR-TB is a worldwide problem; according to the World Health Organisation (WHO), TB is the second most infectious killer after COVID-19, even above HIV and AIDS. With rising resistance to current antibiotics and limited solutions, the urgent discovery of new, effective, and affordable antibacterials with low toxicity is imperative to combat MDR-TB strains. Multidrug-Resistant tuberculosis (MDR-TB), caused by mycobacterium tuberculosis, is resistant to ethambutol (EMB), which has been widely ported worldwide. EMB resistance is caused by mutations in the embB gene, which encodes the arabinosylindoylacetylinositol synthase enzyme. The mutations found in M306L, M3306L + E378A, M306V, and D1024N were utilized as the basis of our protein model. Caryophyllene oxide, Bisabolene, and Trans-caryophyllene are essential components of the medicinal plant *Thymus vulgaris*. Hence, this study will introduce an *in-silico* phytochemical-based approach for discovering novel bacterial agents, exploring the potential of a computational approach in therapeutic discovery. This study focuses on screening all these phytochemicals, Caryophyllene oxide, Bisabolene, and Trans-Caryophyllene, as a potential drug candidate to combat MDR-TB infection through a molecular docking approach. Moreover, the interaction of amino acid analysis, *in silico* pharmacokinetics, compound target prediction, pathway enrichment analysis, and Molecular Dynamics (MD) simulations were conducted for further investigation. Caryophyllene oxide, Bisabolene, and Trans-Caryophyllene also showed a strong binding affinity against these mutations. *in silico* pharmacokinetic analysis highlights the potency as a drug candidate, showing strong Adsorption, Distribution, Metabolism, and Excretion (ADME) properties in combination with low toxicity. This is the first study as per the knowledge of authors for testing these phytochemicals against MDR-TB.

Keywords: MDR-TB, bioactive compounds, molecular docking, molecular dynamics, *Thymus vulgaris*
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Introduction

Tuberculosis (TB) is an infectious disease that mostly affects the lungs and is caused by *Mycobacterium Tuberculosis*, which spreads through the air when infected people cough, sneeze, or spit. According to WHO (2025), an estimated 10.6 million people were ill with TB worldwide, including 5.8 million men, 3.5 million women, and 1.3 million children; TB is present in every country, and all age groups and a total of 1.3 million people died from TB in 2022 including 167 000 people with HIV. MDR-TB remains a public health security threat. Only about 2 in 5 people with drug-resistant TB accessed treatment in 2022. Ending the TB epidemic by 2030 is among the United Nations Sustainable Development Goals (SDGs) aims. Case reports of Mycobacterium Tuberculosis being resistant against the first line drugs such as rifampicin, isoniazid, ethambutol (EMB), Streptomycin, and other drugs have been reported widely. About 3% of all newly diagnosed patients have MDR-TB, and the proportion is higher in the patients who have previously received Anti-tuberculosis treatment, which reflects the failures of the programs that were designed to ensure the complete cure of TB. Host genetic factors can also contribute; inadequate and incomplete treatment is the most important factor leading to the development of MDR-TB (Sharma & Mohan, 2004). Therefore, finding new, effective, and affordable antibacterials with low toxicity is crucial to prevent MDR-TB.

A recent research paper from (Maladan *et al*., 2023) has successfully shown the mutations in the embB gene that encodes for arabinosylindoylacetylinositol synthase. M306L, M306L + E378A, M306V, and D1024N are the protein mutations of the embB gene; also, their paper suggested the amino acid changes that have led to these mutations. Studies have reported different results regarding the mutations at codon 306 on the embB gene (Ruesen *et al*., 2018; Hazbón *et al*., 2005); some studies suggest M306L and M306V mutants do not play any role in the TB resistance towards ethambutol (Bakuła *et al*.2013 and Li *et al*.2020) while many studies predict that M306V mutant is present in both EMB-susceptible and EMB-resistant strains. Other studies suggest that M306L and M306V mutants resist EMB (Sekiguchi *et al*., 2007; Lee *et al*., 2004). Molecular docking is the ability to assign ligand sites on the receptor easily. Computer-based techniques can assist and accelerate the drug discovery process. The binding affinity suggests how strongly the ligand interacts with the binding site of the macromolecules (Mehmood *et al*., 2014). The lower the binding affinity, the stronger the interaction between the ligand and macromolecule.

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Caryophyllene oxide, bisabolene, and Trans-Caryophyllene are essential phytochemicals derived from *Thymus vulgaris* that have shown some potential in combating MDR-TB, often rendering conventional antibiotic treatments (S.Gibbons, 2004). The antimicrobial properties of these compounds serve as valuable for therapies and enhancing their efficacy against resistant strains of *Mycobacterium* Tuberculosis. Caryophyllene oxide, in particular, has shown a strong capacity to inhibit various bacterial strains and disrupt the biofilm formation associated with TB (Joanna *et al*., 2021). Biofilms can protect the bacteria from both host immune response and antibiotic treatments. By interpreting these protective layers, caryophyllene oxide could improve the effectiveness of traditional treatments. Bisabolene and Trans-Caryophyllene further contribute to the antimicrobial actions. Tb triggers a robust inflammatory response; these compounds could help mitigate associated symptoms and improve patient comfort. The anti-inflammatory also aids in reducing tissue damage and promoting recovery. Moreover, the effects of compounds could enhance the overall therapeutic profile of *thymus vulgaris* as a medicinal herb. Integrating these natural compounds into current medicinal practices offers a promising avenue for addressing challenges in infectious disease management today. (Sharifi *et al*., 2017; Rivas *et al*., 2012). This research uses computational methods to evaluate the properties of Caryophyllene oxide, bisabolene, and trans-caryophyllene from *Thymus vulgaris* against MDR-TB.

Methods

We have followed the mutation that is shown by Maladan *et al*. (2023) and it showed the amino acid replacement in a different position of protein structure of arabinosylindoylacetylinositol synthase in MTB shown in Table 1.

Table 1. Mutations found in 7BVF protein structure

Amino Acid Positions	Amino acid	Amino Acid change	Reference
306	Methionine	Leucine, valine, isoleucine	Maladan *et al*. (2021)
328	Aspartic Acid	Tyrosine	Ali *et al*. (2015)
378	Glutamic acid	Alanine	Ali *et al*. (2015)
354	Aspartic acid	Alanine	Ruesen *et al*. (2018)
406	Glycine	Alanine, Aspartic acid	Ruesen *et al*. (2018)
497	Glutamine	Arginine	Lee *et al*. (2020)
1024	Aspartic acid	Asparagine, Threonine	Maladan *et al*. (2021)

MTB mutant embB construction:

The 3D structure of arabinosylindoylacetylinositol synthase in MTB was obtained from the RCSB PDB with PDB ID: 7BVF and only chain A was considered RCSB PDB. Discovery studio 2024 was used for the removal of other chains, removing the water molecules and other unwanted molecules (Biovia *et al*., 2024). The SWISS-MODEL was used for the mutant's structure prediction for the Homology Modelling which was accessed on 19 August 2024 (Waterhouse *et al*., 2018).

Ligand preparation and Molecular Docking

First the ligand was obtained from PubChem in SDF format we have used and in PyRx and minimized and made it ready for the docking. Molecular docking was performed using PyRx Software for blind docking to sort *Thymus vulgaris* essential compounds on the basis of Binding affinity (Trott *et al*., 2010). Later cross checked the autodocking results at CB-Dock2 which has an integrated tool to identify the binding cavities on the proteins. The Grid box size was X: 168.34, Y:146.70, and Z: 175.11 which was determined on the basis of the EMB grid on PDB ID 7BVF as shown in Table 2 (Liu *et al*., 2022).

Table 2. Site of cavities on mutant proteins and its coordinates

Mutation Name	Cavity volume ($Å^3$)	Center (x, y, z)	Cavity size (x, y, z)
7BVF (Standard)	6380	169, 144, 172	22, 30, 30
306M_V	6590	169, 144, 173	22, 30, 30
D1024N	6610	169, 144, 173	22, 30, 30
M306L	6355	169, 144, 173	22, 30, 30
M306L_378A	6355	169, 144, 173	22, 30, 30
M378A	6531	169, 144, 173	22, 30, 30

After completing the HMM for the mutation and validation of the structures using Ramachandran plotting using VADAR and shortlisted the structures on the basis of Z Score that is generated by the ProSA web server that both websites were accessed on 20 August of 2024 (Wiederstein & Sippl, 2007).

ADMET Protocol

For effective and safe drugs exhibit a finely tuned combination of pharmacokinetics, and pharmacodynamics, including the high affinity, potency and selectivity against the molecular target, along with the adequate Adsorption, Distribution, Metabolism, and Excretion (ADMET) (Ferreira *et al*., 2019). We have used SwissADME to know the toxicity of the ligands and test it under different parameters of a drug. Lipinski's Rule of Five is one the most important rules to pass for a drug

In silico toxicology is a test to predict the potential toxicity of chemicals before they are tested in the labs, it aids to meet regulatory requirements by providing the predictions about how the chemicals might interact with biological systems. Toxtree was deployed for that regards (Wicaksono & Parikesit, 2023).

Molecular Dynamics:

We have used online molecular dynamics server CABS-flex 2.0. It was designed to determine analyse the protein flexibility and provide data in RMSF value. It measures the amplitude of structures during the dynamic simulation (Bornot *et al*., 2011). All the parameters were of additional distance restrain, distance restrain generator and advanced simulation options were set as default values (Kuriata *et al*., 2018). MD (Molecular Dynamics) simulation is used to get an RMSF graph which displays the fluctuation of individual residues of a protein complex. The lower the RMSF values the more stable the structure. We have used CABSflex2.0 for the molecular dynamic simulation of the proteins which was accessed

on 16 September 2024 (Kuriata *et al*., 2018; Wicaksono & Parikesit, 2023).

Results

Molecular Docking of Lead Compounds

First, in order to find the best lead compounds as drug candidates, we analysed the blind docking results and found out the best ligands for the MDR-TB mutant proteins as represented in Table 3 and 3D in Figure 1. Binding affinity refers to the strength of the interaction between a molecule, such as a ligand, and its target, typically a protein or receptor. A high binding affinity means that the ligand binds tightly to the target, while a low binding affinity indicates weaker interactions.

Validation of Docking

After getting the docking structures form Auto docking software we need to validate the binding sites and to check that we have use BIOVIA Visual Studio to get the 3D and 2D interaction of the ligands with the mutant proteins with the types of bonds and its distances in Figure 2.

Table 3. Binding affinities of mutant proteins with *Thymus vulgaris* components

Protein Name	Binding Affinity	Ligands ID	Ligand Name
7BVF (Standard)	-7.3	5281515	Trans-Caryophyllene
306M_V	-7.2	1742210	Caryophyllene oxide
D1024N	-7.1	3033866	Bisabolene
M306L	-7.2	5281515	Trans-Caryophyllene
M306L_378A	-7.6	5281515	Trans-Caryophyllene
M378A	-7.6	5281515	Trans-Caryophyllene

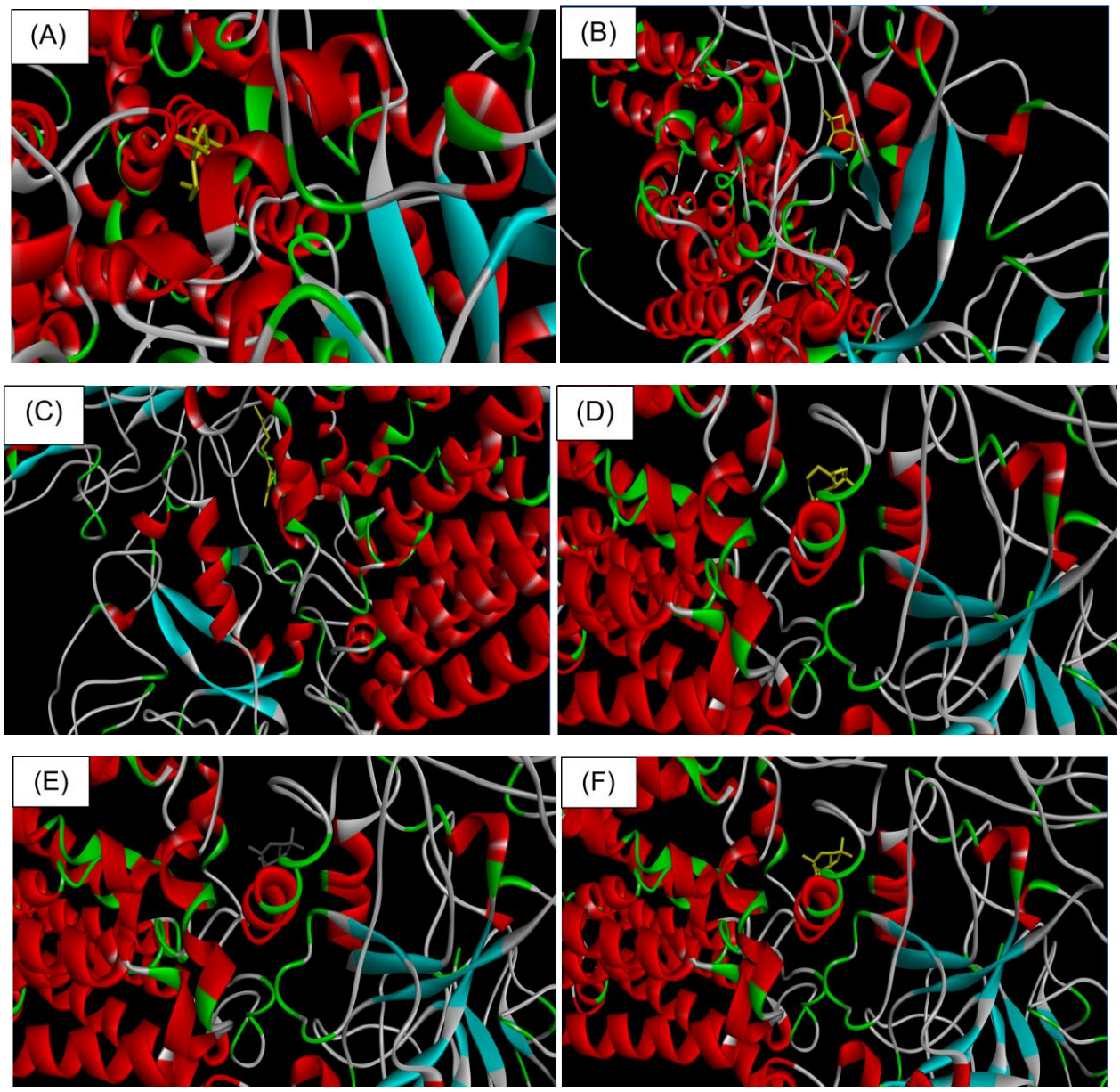

Fig. 1. Showing the interaction between ligand and mutant proteins where red shows helices, cyan shows sheets, green shows binding sites, Grey represents coil (A) 7BVF with Trans-Caryophyllene, (B) 306M_V with Bisabolene, (C) D1024N with Caryophyllene oxide. (D) M306L with Trans-Caryophyllene. (E) M306L_378A with Trans-Caryophyllene, (F) M378A with Trans-Caryophyllene

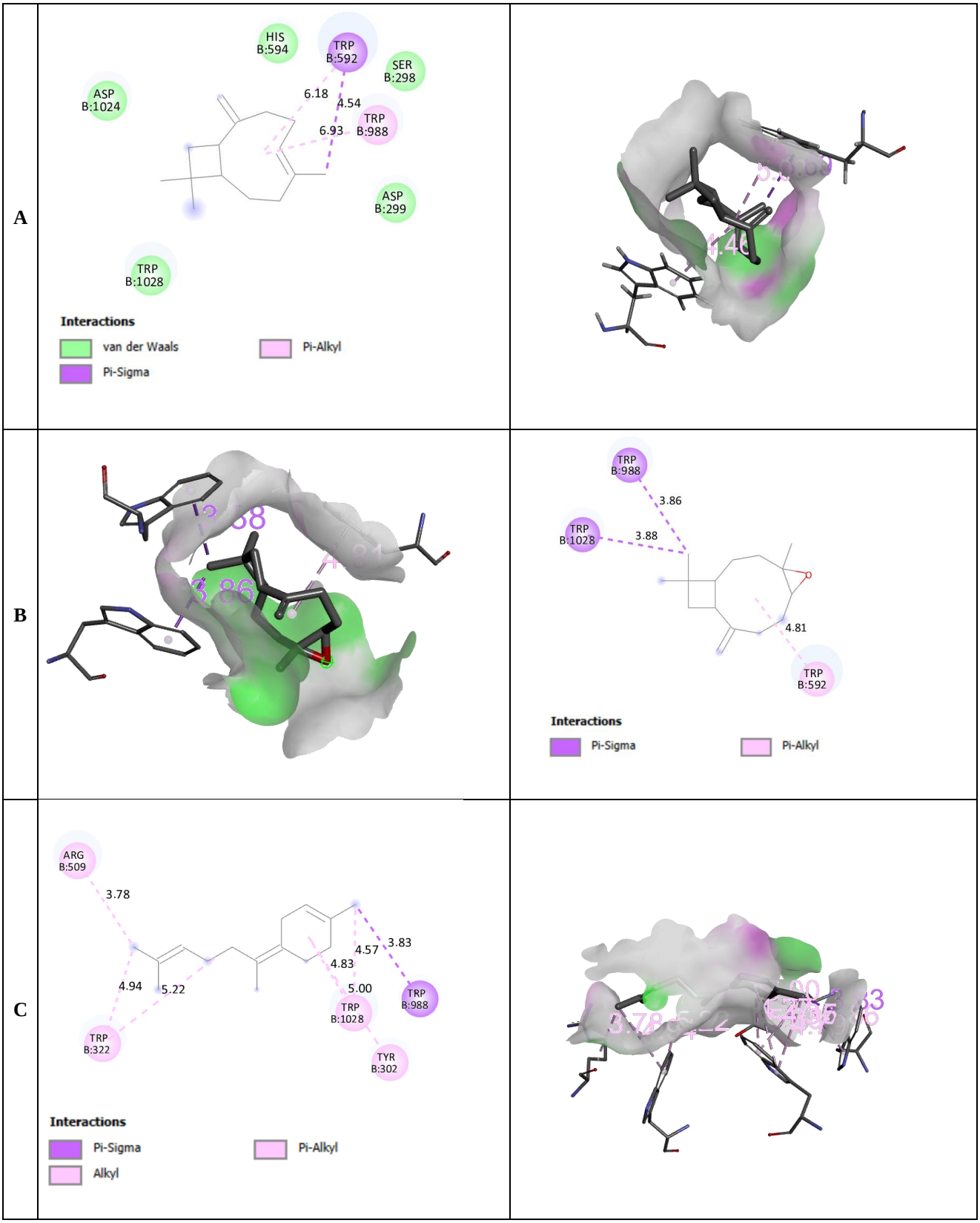

Fig. 2. Showing the 2D and 3D interaction between ligand and mutant proteins, Types of bonds, Distance of Bonds (A) 7BVF with Trans-Caryophyllene, (B) 306M_V with Bisabolene, (C) D1024N with Caryophyllene oxide, (D) M306L with Trans-Caryophyllene, (E) M306L_378A with Trans-Caryophyllene, (F) M378A with Trans-Caryophyllene

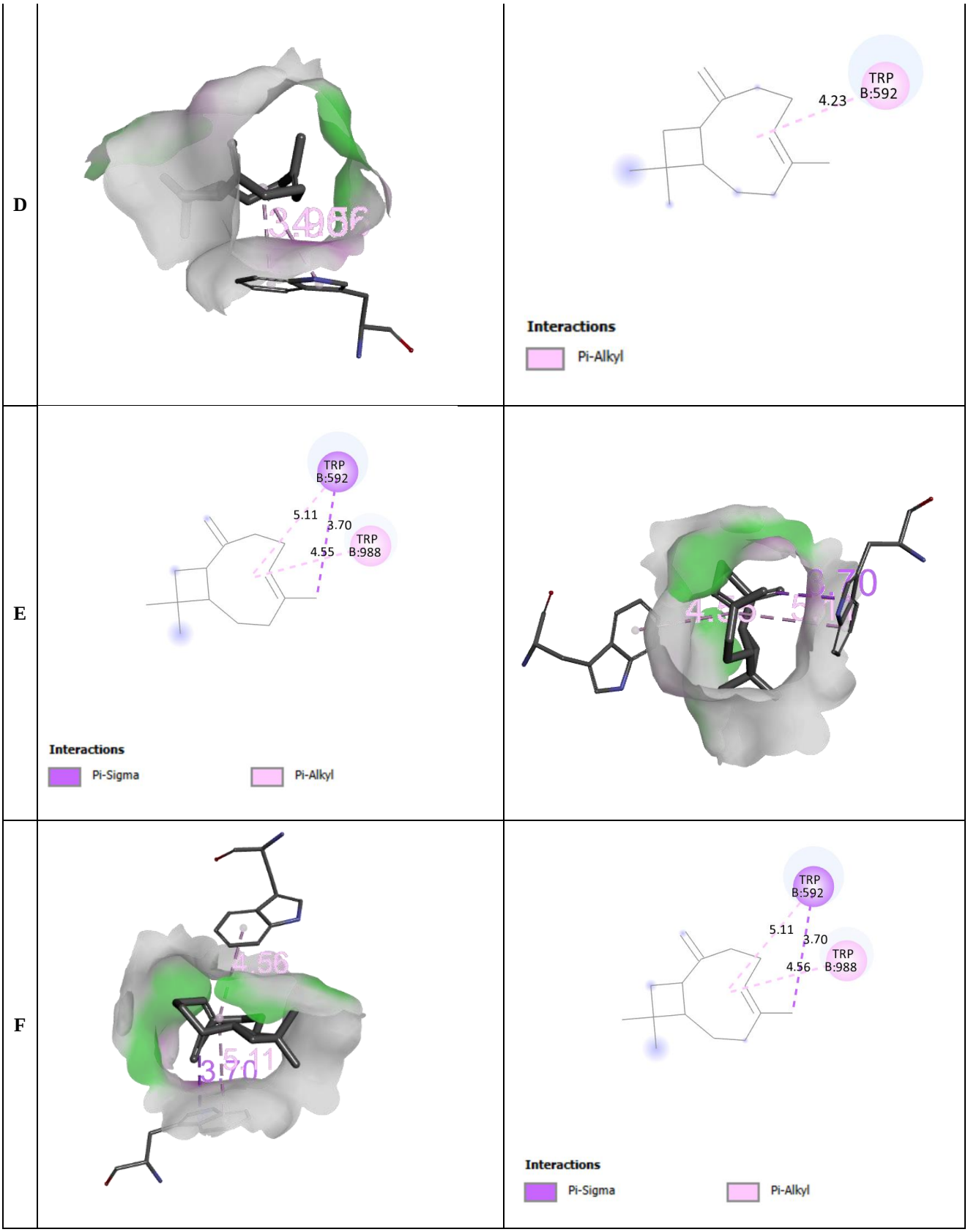

Fig. 2. Showing the 2D and 3D interaction between ligand and mutant proteins, Types of bonds, Distance of Bonds (A) 7BVF with Trans-Caryophyllene, (B) 306M_V with Bisabolene, (C) D1024N with Caryophyllene oxide, (D) M306L with Trans-Caryophyllene, (E) M306L_378A with Trans-Caryophyllene, (F) M378A with Trans-Caryophyllene

ADMET Protocol

SwissADME was accessed 21 August 2024 and shown the results in Table 4 (Diana *et al*., 2017). As the Lipinski's rule of states that an optimum drug should have molecular weight of less than 500 g/mol, the no. of hydrogen bond donor should be less than 5, the No. of hydrogen acceptor bond should be less than 5, the value of MLogP should be less than 5 and the last is the no. of rotatable bonds should be less than 10. Results obtained from SwissADME shows that none of the components violates the 5 laws of Lipinski's, which makes these 3 drug components considerable for this disease (Adebayo *et al*., 2023).

Toxicology Test

Tox tree was accessed on 30 august 2024 and the level of Toxicology is shown in Table 5 (Patlewicz *et al*., 2008). In this study, I have classified the toxicology levels of the ligands based on their degree of toxicity. **Level I** represents the lowest toxicity, indicating that these substances pose minimal risk and are generally considered safe at certain concentrations. These compounds typically have little to no harmful effects. **Level III**, on the other hand, indicates a higher toxicity level, where the substances can cause significant harm, even in relatively small amounts. Therefore, **Level III** corresponds to the highest toxicity, while **Level I** corresponds to the lowest toxicity. This classification system allows for a clear understanding of the relative risk associated with each ligand, with higher levels signifying more dangerous substances.

Molecular Dynamics Simulation:

After getting the results from CABSflex2.0 as shown in Figure 3 we have analysed the output generated as a result. We have checked the RMSF graphs generated by CABSflex2.0 and got to know about the stability of different proteins. In general, each structure has 1024 amino acid residues in it. After analysing the excel sheet generated by CABSflex2.0, In 7BVF there are only 33 residues with more than 3.0 RMSF value that makes it stable, 306M_V has 41 residues over RMSF value of 3.0, D1024N has 40 residues over RMSF value 3.0, M306_L has 41 residues above RMSF value of 3.0, M306L_378A has 38 residues over the value of 3.0, and M378A has only 22 residues with value more than RMSF 3.0. After analysing the results, we can interpret that the M378A has the lowest varying residues which means it is the most stable protein out of all of them (Gunawan *et al*., 2023). The RMSF is considered a crucial indicator to determine the flexibility of protein, in order to measure its stability.

Table 3. Binding affinities of mutant proteins with *thymus vulgaris* components

Protein Name	Binding Affinity	Ligands ID	Ligand Name
7BVF (Standard)	-7.3	5281515	Trans-Caryophyllene
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D1024N	-7.1	3033866	Bisabolene
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M306L_378A	-7.6	5281515	Trans-Caryophyllene
M378A	-7.6	5281515	Trans-Caryophyllene

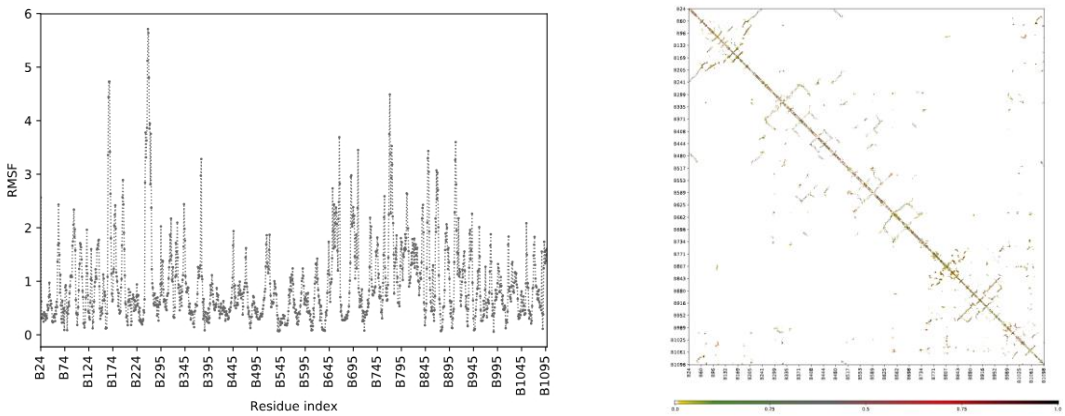
Table 4. Analysis of Drug on Lipinski's Rule of Five

Ligand ID	M. weight (g/mol)	No. Hydrogen acceptors	No. Hydrogen donors	MlogP	Rotatable Bonds
5281515	204.35	0	0	4.63	0
1742210	220.35	1	0	3.63	0
3033866	204.35	0	0	4.53	3

Table 5. Levels of toxicology of different ligands.

Ligand ID	Level of Toxicology
5281515	Level I
1742210	Level III
3033866	Level I

A.



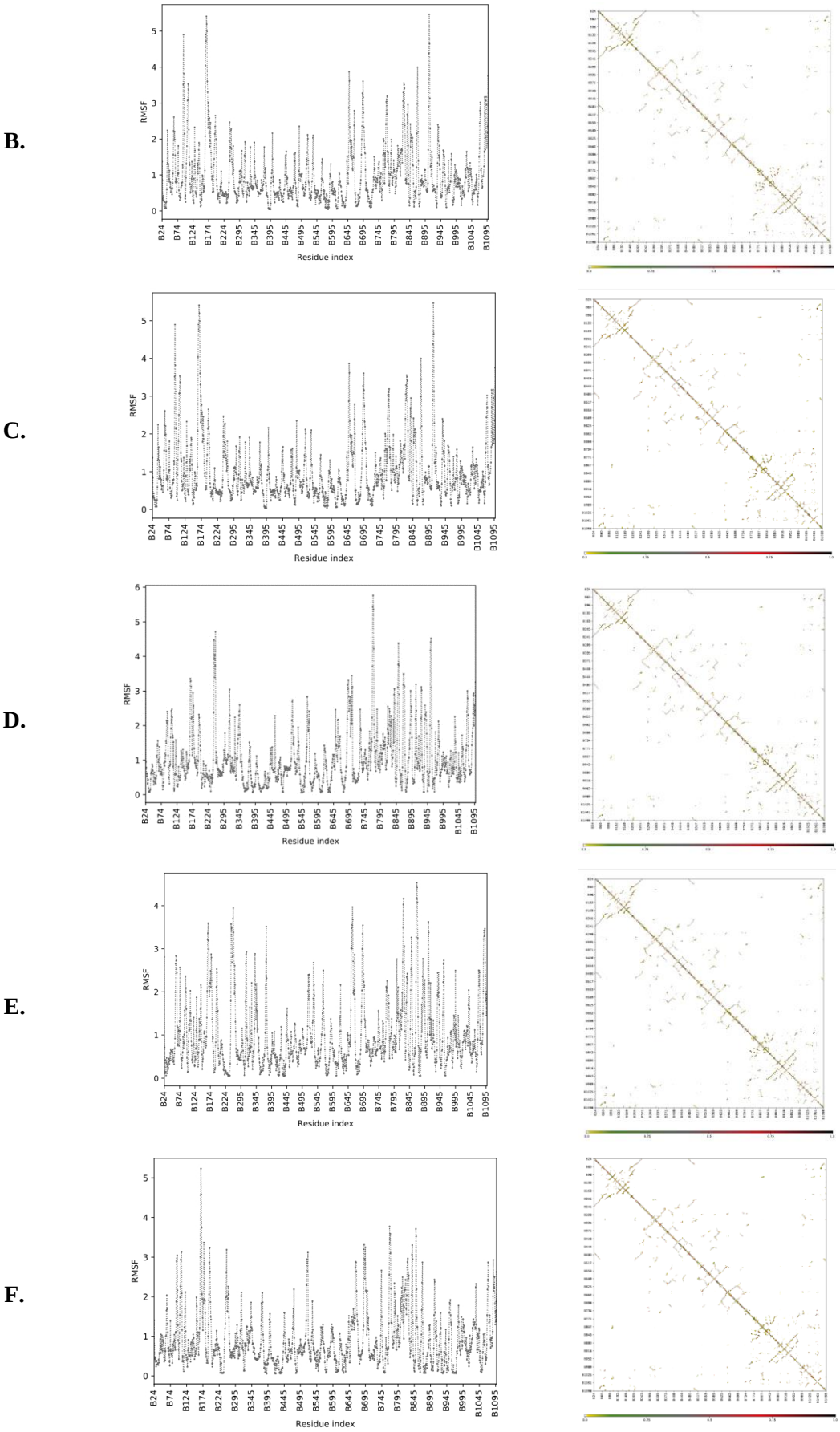


Fig. 3. Showing RMSF Values of interaction between ligand and mutant proteins (A) 7BVF with Trans-Caryophyllene, (B) 306M_V with Bisabolene, (C) D1024N with Caryophyllene oxide, (D) M306L with Trans-Caryophyllene, (E) M306L_378A with Trans-Caryophyllene, (F) M378A with Trans-Caryophyllene

Discussion

This study demonstrates the potential of *Thymus vulgaris* Phytochemicals, mainly Caryophyllene oxide, bisabolene, and Trans-Caryophyllene, as promising inhibitors of the MDR-TB proteins. The docking result suggests that trans-caryophyllene and caryophyllene oxide are the most potent inhibitors based on binding affinity from docking results. It was considered to take only the 3 best ligands from the components of *Thymus vulgaris* as there are more than 10 components found in *thymus vulgaris*, as shown in this paper (Shabnum *et al*., 2011; Padmi *et al*., 2022).

After docking between all the compounds and the mutant proteins, we learn about the binding affinity of each compound with the mutant proteins, which creates resistance to the ethambutol drug. The binding affinity is promising, which makes these 3 components considerable for future research. These components, bisabolene bisabolene (Jin *et al*., 2022), Trans-Caryophyllene (Hu *et al*., 2022), and Caryophyllene oxide (Kanokmedhakul S *et al*., 2007), have already shown antimicrobial properties against S.aureus, E.coli, and Monilia albicanswhich makes us curious to explore about them. After ADME-Tox results, we have found out that there is only one compound out of 3 that is showing a high level of hazard to the body because of two membered rings, which can be changed using some structural change in the component or by the experimental work in the labs (Boniface *et al*., 2017; Aini *et al*., 2022).

These compounds are supported by their pharmacokinetic properties evaluated by SwissADME, showing none violate Lipinski's Rule of five. In contrast, Caryophyllene oxide shows some level of toxicity while the binding affinity shows as a lead component and for further refinement. The MD simulation result also highlights the stability of the ligand-protein complex, particularly the M378A mutant protein, which has the lowest RMSF value as it is still fall into the acceptable threshold. It also indicating stable interaction. This study builds on the understanding of the embB mutations and suggests that specific compounds from *Thymus vulgaris* could be explored as new avenues for treatment. The future aspect is to validate these phytochemicals through in vitro and in vivo experiments to confirm their effects against MDR-TB. Exploring synergistic combinations with existing TB drugs can be effective therapeutic strategy.

Conclusion

This study has shown that these components, Caryophyllene oxide, Bisabolene, and Trans-Caryophyllene from *Thymus vulgaris*, have the potential to inhibit 7BVF and its mutant protein of MDR-TB. The gastrointestinal tract, soluble in water and bioavailable, readily absorbs all ligands. All of the ligands have passed the ADME test. Although Caryophyllene oxide has shown toxicology, other drugs, such as Trans-Caryophyllene and Bisabolene, have no side effects. The drug can be optimized in the wet lab to remove these effects. However, since this is*in silico* research, further wet lab validations, such as *in vivo* or *in vitro* experiments, are needed to approve these results.

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