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Discovery of Plant-Based Inhibitors of *Mycobacterium tuberculosis* Shikimate Kinase: An In Silico Study

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ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a critical global health issue with significant cases and deaths reported annually. The emergence of drug-resistant TB strains like multidrug- and extensively drug-resistant (M/XDR) TB exacerbates the challenge, necessitating more efforts in drug discovery approaches. One promising target for drug development is Shikimate Kinase (MtSK), a key enzyme in the shikimate pathway that is essential for the biosynthesis of aromatic amino acids and other key metabolites. This study explored the potential inhibitory activity of 1137 plant-derived compounds from the HerbalDB database against MtSK through pharmacophore modeling, molecular docking, molecular dynamics simulation, and ADMET analysis. Chlorogenic acid and sinigrin emerged as the top candidates, exhibiting strong binding affinities and favorable interactions with key residues in the MtSK active site. Molecular dynamics simulations confirmed the stability of these compounds within the active site, while ADMET and druglikeness evaluations further highlighted their potential as drug candidates. These findings suggest that plant-derived compounds, particularly chlorogenic acid and sinigrin, could be developed as inhibitors of MtSK, contributing to the fight against drug-resistant TB.

Keywords

tuberculosis, shikimate kinase, inhibitor, plant-derived compound

INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis*, is recorded as the world's deadliest infectious disease with more than 10 million cases and 1.3 million deaths per year. In 2022, WHO reported an increase in tuberculosis cases to 10.6 million after recording 10.3 million cases and 10 million cases in 2020 and 2021. A further problem, approximately 410,000 people developed multidrug- or rifampicin-resistant tuberculosis (MDR/RR-TB) in the same year. First-line tuberculosis treatment uses four-drug regimens, including rifampicin, isoniazid, ethambutol, and pyrazinamide, with a cure rate of 88%. For drug-resistant tuberculosis, treatment with bedaquiline, pretomanide, linezolid, and moxifloxacin has a lower cure rate of 66% (World Health Organization 2023). More strategies and efforts are needed to discover new drugs to address the resistance problem and increase the cure rates.

Targeting metabolic pathways of pathogens that are absent in mammals is a strategic step in drug discovery research to obtain specific inhibitors and potentially reduce the risk of toxicity (Pereira et al. 2004). The shikimic acid pathway in bacteria, algae, fungi, and higher plants is essential for the biosynthesis of precursors of many important metabolites such as aromatic amino acids, folate, and quinones (Bentley 1990). On the other hand, mammals do not have this pathway because most aromatic compounds are obtained from nutrition. The shikimic acid pathway uses carbohydrates to synthesize aromatic compounds through seven steps involving several enzymes, with chorismate as the final product (Nunes et al. 2020).

Shikimate kinase (SK), EC 2.7.1.71, is an enzyme that catalyzes the fifth step in the shikimate pathway. This enzyme catalyzes phosphoryl transfer from ATP to shikimate to produce ADP and shikimate-3-phosphate as products (Hartmann et al. 2006). In *M. tuberculosis*, SK is encoded by the *aroK* gene, producing a protein consisting of 176 amino acids with a weight of 18.6 kDa. SK is a nucleoside monophosphate kinase (NMP kinase) family member that undergoes significant conformational changes during catalysis. This enzyme consists of three domains: CORE, which contains the P-loop for phosphate binding; the LID domain, which undergoes structural changes upon substrate binding; and the NMP binding domain, which plays a role in substrate recognition and binding (Nunes et al. 2020). Shikimate kinase is essential for the survival of *M. tuberculosis*; therefore, its inhibition could be a strategic step in drug discovery to combat tuberculosis (Parish and Stoker 2002).

Plants, as a source of a wide range of natural products with various therapeutic properties, are constantly explored to develop novel drugs (Newman and Cragg 2020). The diversity of chemical structures makes it attractive to explore their biological activities further. This biological activity is related to the compounds' ability to influence physiological functions and chemical pathways (Koehn and Carter 2005). Furthermore, plants' evolutionary ability to produce compounds for pathogen and pest defense has a high potential for preventing pathogen resistance (Dias et al. 2012). The discovery and use of natural compounds derived from plants, as in this study, can provide hope for new therapies to overcome various health challenges, including infectious diseases.

This study focuses on the potential inhibitory activity of 1,137 compounds from the HerbalDB database against Mycobacterium tuberculosis Shikimate kinase (MtSK). This investigation aims to examine the binding mechanisms and interactions of these compounds with MtSK through pharmacophore screening, molecular docking, and molecular dynamics simulations. Additionally, ADMET studies and druglikeness evaluations were performed to assess the pharmacokinetic properties and suitability of the compounds as drug candidates. Targeting MtSK is crucial due to its essential role in the bacterial survival pathway and the absence of homologous proteins in humans, making it a promising target for antitubercular drug development.

Material and Methods

Structure-based pharmacophore model generation

The structure-based pharmacophore model was developed by analyzing the essential interaction pattern between the MtSK protein and the ligand. This study utilized the crystal structure of MtSK bound to shikimate and ADP (PDB: 2IYQ) for pharmacophore model generation. LigandScout 4.4.9 (Austria, Europe) software was employed to construct the pharmacophore model, which typically encompasses features such as hydrogen bond acceptors, hydrogen bond donors, and hydrophobic interactions within the catalytic site of the protein (Wolber and Langer 2005). The shikimate binding site on the protein was specifically targeted for model development instead of the ADP binding site, aiming to design inhibitors with higher selectivity for MtSK, considering the prevalence of ATP- or ADP-binding motifs across kinases.

Pharmacophore model validation

The pharmacophore model was validated using LigandScout with 9 known inhibitors of Mycobacterium tuberculosis Shikimate kinase (MtSK) and 253 decoy compounds. The model was generated based on key pharmacophoric features from the inhibitors, such as hydrogen bond donors, acceptors, and hydrophobic interactions. Virtual screening was performed, where both inhibitors and decoys were aligned to the pharmacophore, and fitness scores were calculated. The model's performance was evaluated using a Receiver Operating Characteristic (ROC) curve, with the Area Under the Curve (AUC) used to assess its ability to distinguish between true inhibitors and decoys.

Molecular docking

The protein structure was retrieved from Protein Data Bank (PDB: 2IYQ) and the acquisition of ligand structures from PubChem, followed by their 3D optimization using MarvinSketch. Ligands demonstrating high pharmacophore fit scores were prioritized for subsequent molecular docking procedures. The receptor (protein) and each ligand were prepared using AutoDock and saved in pdbqt format. AutoDock 1.5.7 was employed for the docking simulations, with the receptor treated as rigid and each ligand as flexible. To validate the docking accuracy, a redocking procedure of the native ligand from 2IYQ was conducted. Interacting residues were analyzed and visualized using LigPlot(+), ensuring a comprehensive assessment of binding interactions.

Molecular dynamic

Molecular dynamic simulation was conducted using YASARA Dynamic ver 22.8.22 (YASARA Biosciences GmbH, Vienna, Austria) (Land and Humble 2018). The simulation ran for 150000 ps, or 150 ns, with a snapshot captured every 100 ps. A cube waterbox was used as the simulation cell, and AMBER14 (Wang et al. 2004) force fields were used in the process. The energy was minimized using the steepest descent minimization algorithm prior to the commencement of the simulation (Piotto et al. 2017). Numerous physiological parameters, including pH 7.4, temperature 298 K, 0.9% NaCl ions and water density 0.997 g/L (Dickson et al. 2014) all contributed to the sample's viability. Following the completion of the simulation, the following commands were executed: md_analyze.mcr to obtain the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Dynamic Cross-Correlation Matrix

(DCCM); md.analyzebindenergy.mcr was then used to obtain the binding energy value (Krieger and Vriend 2015; Rasul et al. 2023).

ADMET study and druglikeness evaluation

The ADMET analysis was conducted using ADMETLab 2.0 (Xiong et al. 2021) to evaluate the Absorption, Distribution, Metabolism, Excretion, and Toxicity profiles of selected compounds. This analysis provided crucial insights into the compounds' pharmacokinetic behavior and potential safety as drug candidates. Additionally, druglikeness and bioavailability characteristics were assessed using SwissADME (<http://www.swissadme.ch/>). This tool evaluated key properties such as Lipinski's rule of five, bioavailability score, and solubility, ensuring the compounds' suitability for further development as orally administrable drugs.

Result and Discussion

Pharmacophore model

Structure-based pharmacophore modeling is an essential method in drug discovery, aiding in the identification of key molecular features critical for a compound's biological activity. This study examined the interaction of shikimate with its binding site in the *Mycobacterium tuberculosis* shikimate kinase (MtSK) structure (Figure 1). The pharmacophore model generated from this interaction provides several significant insights.

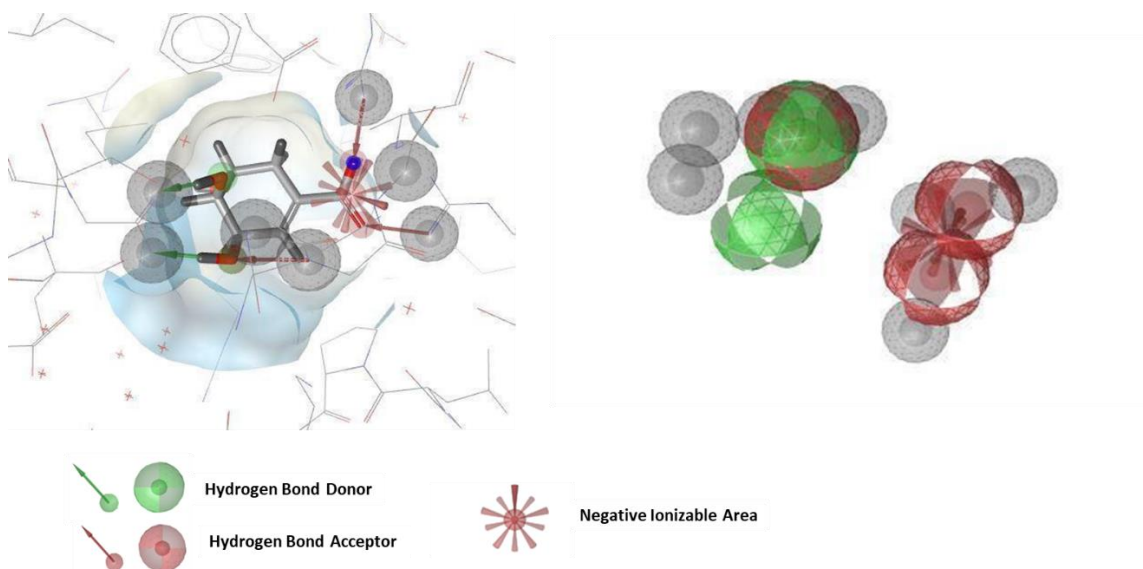


Figure 1. Structure-based pharmacophore model based on key interaction of MtSK-shikimate

Four hydrogen bond acceptor features involving Gly80, Arg136, and Arg58 residues have been identified, indicating that these sites are essential for shikimate binding. Hydrogen bonds are essential for establishing stable interactions between the ligand and the protein, hence increasing binding affinity (Fersht 1987). Two hydrogen bond donor features were observed, both involving the Asp34 residue. This interaction is significant as it contributes to the shikimate's binding affinity and specificity to the *MtSK* active site. One negative ionizable area was identified in the vicinity of the binding site. This feature corresponds to regions where negatively charged groups on shikimate interact with positively charged residues or regions within the binding site, further stabilizing the complex (Ojeda-May 2021).

The identified pharmacophore features are consistent with the known active site residues of *MtSK*. The involvement of Gly80, Arg136, Arg58, and Asp34 indicates that these residues are critical for the binding and activity of shikimate. These interactions highlight the importance of hydrogen bonding and, notably, electrostatic interactions in the stabilization and function of the shikimate-*MtSK* complex (Pereira et al. 2004).

Pharmacophore model validation

The pharmacophore model was validated using a dataset of 9 known *MtSK* inhibitors and 253 decoys. The performance of the pharmacophore model was analyzed using the ROC curve (Figure 2) generated from the simulation test on the dataset. The model successfully identified three hits, including two true positives and one false positive, meaning that 2 were actual inhibitors and 1 was a decoy compound.

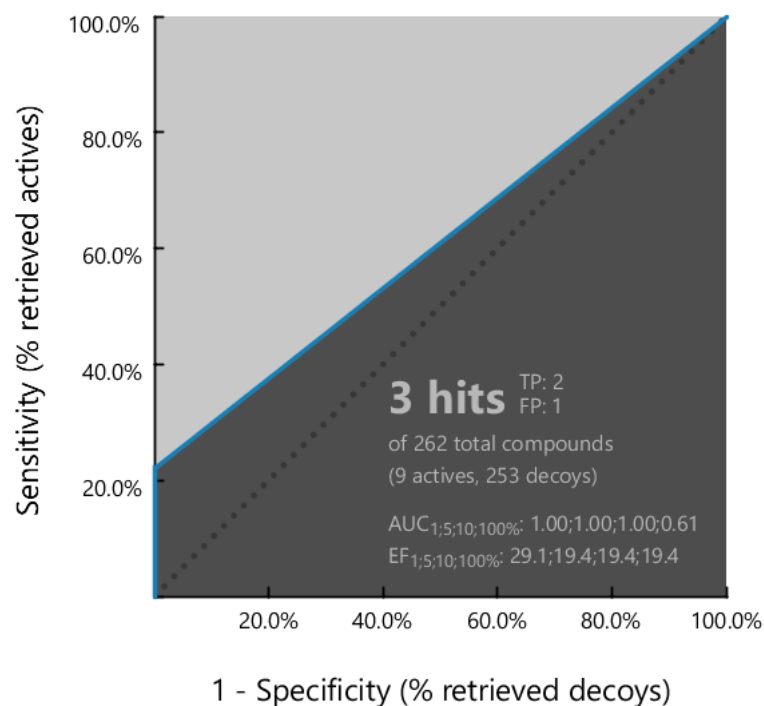


Figure 2. The receiver operating characteristics (ROC) validation curve of pharmacophore model

The AUC_{1;5;10;100%} values were 1.00; 1.00; 1.00; 0.61, indicating the model's performance at the top 1%, 5%, 10%, and 100% levels. The AUCs of 1, 5, and 10% showed perfect values (1.00), meaning the model was very good at identifying active compounds in the top small percentage. The AUC of 100% was 0.61, which was lower but still above the random value (0.5), indicating acceptable performance for the entire dataset. In addition, the EF_{1;5;10;100%} values are 29.1; 19.4; 19.4; 19.4, indicating enrichment factors at several levels of compound percentage. The high EF value at 1% suggests that the model effectively selects active compounds at the top level. The 5% to 100% EF remains consistent at 19.4, indicating stable enrichment after the initial subset.

The model's ability to maintain high accuracy with minimal false positives among the top-ranked compounds supports its application in targeted virtual screening. This validation demonstrates the pharmacophore model's effectiveness for early identification of MtSK inhibitors, showcasing a strong enrichment factor and robust accuracy.

Pharmacophore virtual screening

This work performed pharmacophore-based virtual screening on a database of 1377 compounds derived from Indonesian medicinal plants, available in HerbalDB (Syahdi et al. 2019). The objective was to discover compounds that display pharmacophore characteristics resembling the interactions seen between shikimate and the molecular residues in *MtSK*. A total of 30 compounds were found to exhibit a high degree of similarity with the established pharmacophore model during the virtual screening analysis.

Among the identified hits, the top 10 compounds were further analyzed based on their pharmacophore-fit scores, which quantify the degree of similarity between the compounds and the pharmacophore model. Table 1 summarizes these top 10 hit compounds, including their names, pharmacophore-fit scores, and sources.

Table 1. The top-10 of hit compounds from SBDD method

No.	Compound name	Pharmacophore-Fit Score	Source
1	8- Hydroxyapigenin 8-(2'',4''-disulfatoglucuronide)	75.58	<i>Helicteres isora</i> (Yanuar et al. 2014)
2	1-Caffeoyl-beta-D-glucose	65.74	<i>Balanophora sp</i> (Wang et al. 2012)
3	Epigallocatechin 3,3'-di-O-gallate	65.00	<i>Camellia sinensis</i> (Paiva et al. 2022)
4	Chlorogenic acid	57.84	<i>Coffea sp</i> (Purwoko et al. 2022)
5	Dihydrodehydrodiconiferyl alcohol	57.21	<i>Saussurea medusa</i> (Zhao et al. 2017)
6	Genistein	57.03	<i>Soybean</i> (Wang and Murphy 1994)

7	Guaijaverin	56.78	<i>Psidium guajava</i> (Ravi and Divyashree 2014)
8	Sinigrin	56.76	<i>Brassica nigra</i> (Mazumder et al. 2016a)
9	Isoetin 7-glucoside	56.28	<i>Hispidella hispanica</i> (Harborne 1978)
10	Danielone	56.26	<i>Carica papaya</i> (Echeverri et al. 1997)

Molecular docking

The AutoDock 1.5.7 software was used to conduct a molecular docking simulation. To verify the accuracy of the docking parameters and software utilized, it is necessary to perform a redocking of shikimate (SKM) to the protein structure (PDB ID: 2IYQ). The outcome of redocking is presented in Table 2. The examination evaluates whether the SKM ligand can bind to its natural starting location by assessing two factors: the RMSD value and the interacting residues. Following three rounds of redocking, it was discovered that the ligand could reattach to its precise initial location, as indicated by the RMSD value ($<2 \text{ \AA}$). Furthermore, the relationship between the examined SKM and protein structure was mediated by four specific residues engaged in hydrogen bonding. These residues include Asp34, Arg58, Gly80, and Arg136. This finding is similar to previous studies regarding the residues that are involved in the interaction between SKM and MtSK (Hartmann et al. 2006). Thus, the docking parameters and software used were appropriate for the docking task.

Table 2. The result of redocking SKM to MtSK (PDB ID: 2IYQ)

Trial	Binding Pose	RMSD	Binding Affinity	Involved Residues	
				Hydrogen Bond	Hydrophobic Bond

1	1	0.63	-7.45	Asp34, Arg58, Gly80, Arg136	Pro11, Ile45, Phe57, Gly79, Gly81, Arg117, Pro118, Leu119
2	1	0.83	-7.4	Asp34, Arg58, Gly80, Arg136	Pro11, Ile45, Phe57, Gly79, Gly81, Arg117, Pro118, Leu119
3	1	0.63	7.47	Asp34, Arg58, Gly80, Arg136	Pro11, Ile45, Phe57, Gly79, Gly81, Arg117, Pro118, Leu119

The molecular docking results (Table 3) indicate varying degrees of binding affinities and inhibition constants for the tested compounds against the MtSK. The native ligand, shikimate, exhibited a binding affinity of -7.45 kcal/mol and an inhibition constant (K_i) of 3.480 μ M, serving as a reference for comparing other compounds.

Table 3. Molecular docking results of pharmacophore screening hits

No.	Compound	Binding Affinity (ΔG), kcal/mol	Involved Residues	
			Hydrogen Bond	Hydrophobic Bond
	Shikimate (native ligand)	-7,45	Asp34, Arg58, Gly80, Arg136	Pro11, Ile45, Phe57, Gly79, Gly81, Arg117, Pro118, Leu119
1	8- Hydroxyapigenin 8- (2'',4''-disulfatoglucuronide)	-5,17	Gly12, Ser16, Asp32, Asp34, Arg58, Gly80, Glu61, Gly81	Pro11, Lys15, Ile45, Phe49, Gly79, Val116,

			Pro118, Leu119, Leu132
2	1-Caffeoyl-beta-D-glucose	-5,19 Ser16, Arg58, Gly81, Arg136	Pro11, Asp34, Val35, Ile45, Phe49, Gly79, Gly80, Val116, Arg117, Pro118, Leu119
3	Epigallocatechin 3,3'-di-O-gallate	-6,43 Ser16, Asp32, Glu38, Arg58, Gly81, Asn114, Val116	Pro11, Lys15, Asp34, Val35, Ile45, Phe49, Phe57, Ser77, Leu78, Gly79, Gly80, Arg117, Pro118
4	Chlorogenic acid	-9,09 Gly12, Ser16, Asp34, Arg58, Gly81, Arg117, Arg136	Pro11, Lys15, Ile45, Phe49, Phe57, Gly79, Gly80, Pro118, Leu119
5	Dihydrodehydrodiconiferyl alcohol	-5,95 Arg58, Gly81, Val116, Arg136	Asp34, Ile45, Phe57, Glu61, Gly79, Gly80, Arg117, Leu119
6	Genistein	-7,44 Ser16, Leu119	Pro11, Lys15, Asp34, Phe49, Gly80, Arg117, Pro118,
7	Guaijaverin	-7,18 Ser16, Arg58, Gly80, Val116, Arg136	Pro11, Gly12, Lys15, Asp34,

			Ile45, Gly81, Pro118, Leu119
8	Sinigrin	-8,52	Asp34, Arg58, Gly80, Leu119
			Pro11, Lys15, Ile45, Phe49, Phe57, Gly79, Gly81, Val116, Arg117, Pro118, Leu119
9	Isoetin 7-glucoside	-5,81	Lys15, Ser16, Asp32, Asp34, Gly80, Leu119
			Pro11, Val35, Ile45, Phe49, Gly79, Arg117, Pro118
10	Danielone	-6,03	Lys15, Arg58, Arg117, Arg136
			Pro11, Asp34, Phe57, Glu61, Gly79, Gly80, Gly81, Leu119, Leu132

Among the tested compounds, chlorogenic acid showed the highest binding affinity with a ΔG of -9.09 kcal/mol and the lowest inhibition constant of 0.219 μM . Chlorogenic acid forms crucial hydrogen bonds with key residues in the MtSK active site, including Gly12, which stabilizes the molecule near the site entrance; Asp34, a catalytic residue suggesting potential interference with the enzyme's function; and Gly81, which supports structural stability. It also interacts with Arg58, Arg117, and Arg136, whose multiple hydrogen bonds are critical in stabilizing chlorogenic acid and blocking substrate access (Figure 3). Additionally, chlorogenic acid engages in hydrophobic interactions with Pro11, Lys15, Ile45, Phe49, and Phe57, creating a hydrophobic pocket that further anchors the molecule, while Gly79 and Gly80 provide further stabilization through non-polar interactions. This suggests that chlorogenic acid has a robust interaction with the active site of MtSK, making it a promising candidate for enzyme inhibition. Previous studies have demonstrated that chlorogenic acid exhibits antioxidant and antimicrobial

properties, further bolstering our optimism about its potential as an effective inhibitor (Farah and Donangelo 2006; Kabir et al. 2014).

Sinigrin also demonstrated significant potential with a binding affinity of -8.52 kcal/mol and an inhibition constant of 0.568 μM . Sinigrin forms essential hydrogen bonds with key MtSK residues, including Asp34, a catalytic residue also targeted by other top-performing inhibitors, and Arg58, which engages in hydrogen bonding, suggesting that sinigrin may disrupt the enzyme's function (Figure 3). Additionally, Gly80 and Gly81 provide a flexible yet stabilizing environment that strengthens sinigrin's binding affinity. Hydrophobic interactions with residues such as Pro11, Lys15, Ile45, Phe49, Phe57, and Leu119 create a hydrophobic network that locks sinigrin into the active site. Sinigrin's sulfur-containing functional groups and glucosinolate backbone further enhance its ability to bind MtSK. The interaction with critical residues like Asp34 and Arg58 is particularly significant, as it may block substrate access or alter enzyme conformation, contributing to its potential as an inhibitor. Sinigrin is a glucosinolate found in cruciferous vegetables and is known for its anticancer and antimicrobial properties (Mazumder et al. 2016b).

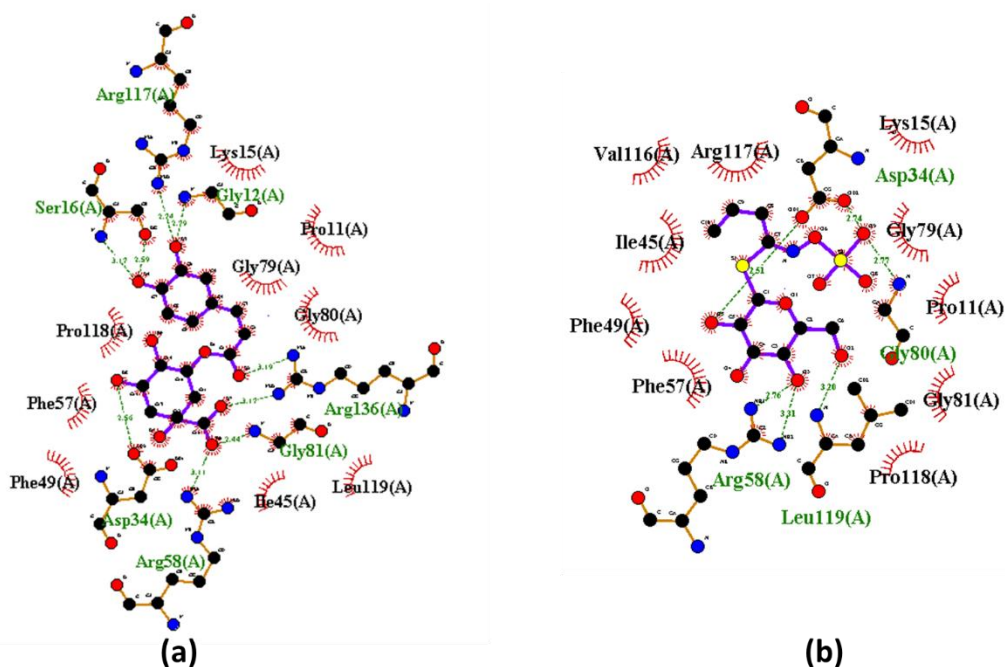


Figure 3. Visualization of interaction of MtSK-chlorogenic acid (a) and MtSK-sinigrin (b)

Both chlorogenic acid and sinigrin have demonstrated strong binding affinities and interactions with key residues in the MtSK active site, making them promising candidates for

further evaluation. To better understand their dynamic behavior and stability in a biological environment, these compounds will be advanced to the molecular dynamics (MD) simulation stage. This step will allow us to assess their structural flexibility, binding stability over time, and their interaction profiles under physiological conditions, providing deeper insights into their potential efficacy as MtSK inhibitors.

Molecular dynamic analysis

The best two compound candidates, chlorogenic acid and sinigrin, as well as the native structure for comparison, which had shikimate (SKM) and ADP inside the structure (PDB ID: 2IYQ), were considered for 150 ns MD simulation for further analysis of the docking results. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), molecular mechanics poisson-boltzmann surface area (MM-PBSA) energy and dynamic cross-correlation matrix (DCCM) were investigated for MD simulation trajectory analysis.

The sinigrin-MtSK complex exhibited a RMSD pattern at the start of the simulation, with an average value of 1.83 ± 0.17 Å. The RMSD reached its greatest value at 2.403 Å. After 65 nanoseconds of simulation, the figure entered an equilibrium phase and maintained a steady RMSD profile until the completion of the experiment, as shown in Figure 4a. The chlorogenic acid-MtSK complex exhibited an unstable RMSD pattern throughout the simulation, with the maximum point reaching 4.02 Å and an average of 2.02 ± 0.42 Å at the end of the simulation. This figure does not appear to reach the equilibrium phase during the simulation. Both mean values exceeded the RMSD of the SKM-MtSK complex, which was 1.46 ± 0.18 Å. The SKM-MtSK complex, which serves as the standard complex in this study, demonstrated a consistently stable RMSD throughout the simulation.

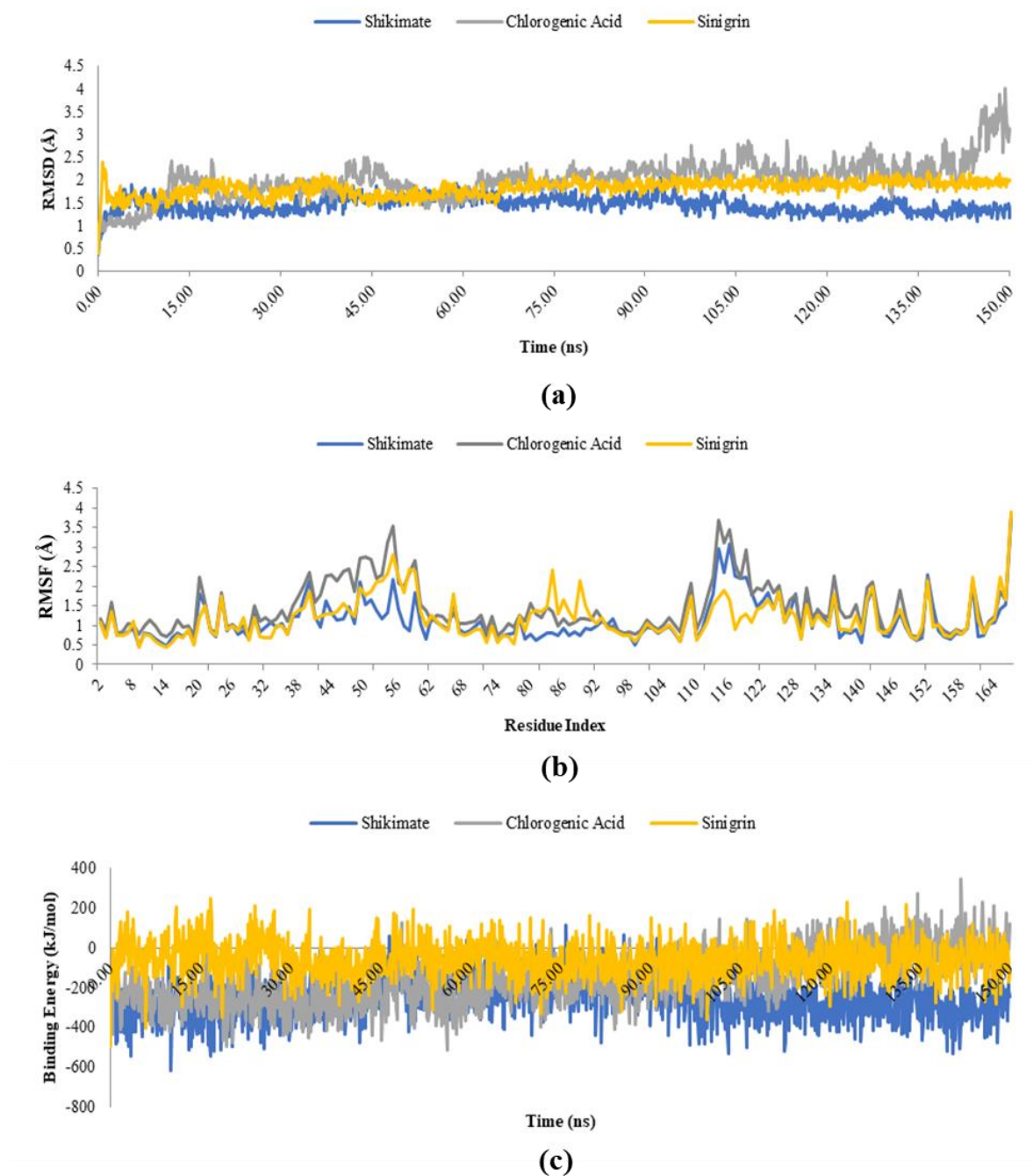


Figure 4. Molecular dynamics simulation Analysis of MtSK protein with selected target complex. (a) RMSD C- α value; (b) RMSF value; and (c) binding energy value

The substrate shikimate interacts with a pocket created by specific residues, including Asp34, Arg58, Gly80, and Arg136, through hydrogen/ionic interactions. This interaction was

simulated using molecular dynamics (Figure 5). Specifically, Arg58 and Arg136 consistently form stable interactions with the carboxyl group of SKM across the entire simulation period. Meanwhile, during the simulation, Asp34 consistently interacted with two hydroxyl groups of shikimate, as well as Gly80. Subsequent to these interactions, there is a breakage at 50 nanoseconds, resulting in a modest disturbance in the stability of the complex, as observed in its RMSD (Figure 4a). However, these two bonds ultimately reemerge at around 95 nanoseconds, further confirming the stability of the complex and remaining intact until the simulation concludes. This finding strongly correlates with the results of prior research, which emphasize the significance of these residues in the active region of *MtSK* as catalytic residues (Hartmann et al. 2006).

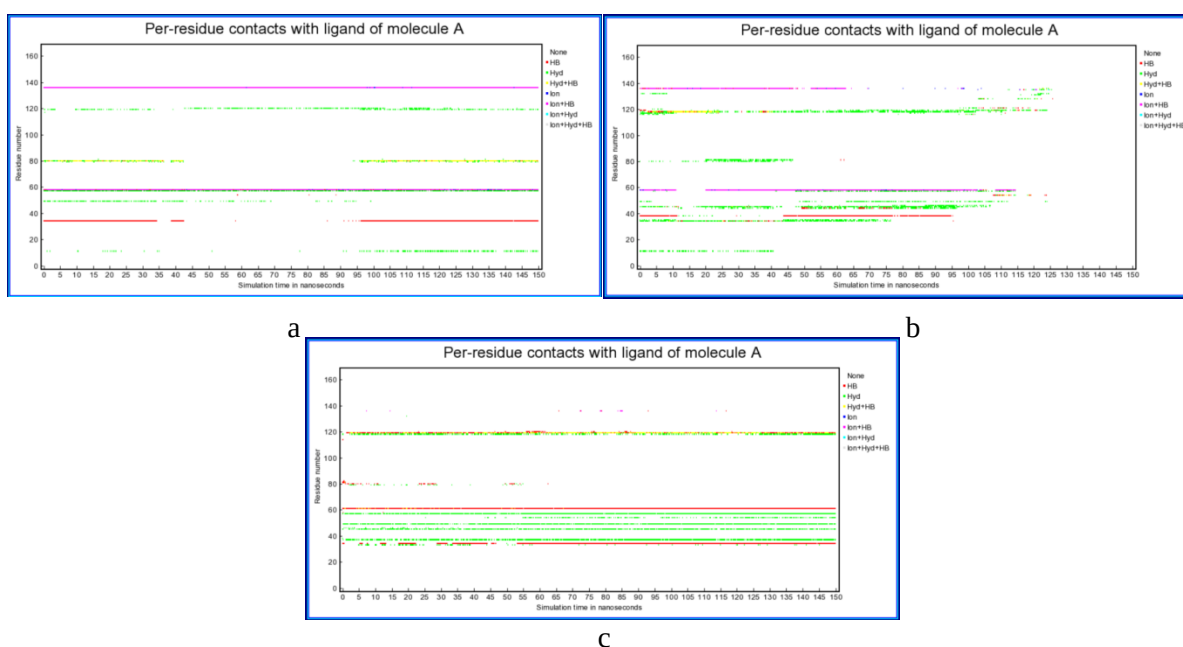


Figure 5. Per residue contact between ligand and: a) native inhibitor (SKM); b) chlorogenic acid; c) sinigrin. Color indication: hydrophobic bond (Green); hydrogen bond (Red), ionic+hydrogen bond (Purple)

The molecular dynamic study also revealed that sinigrin interacted with the active site of *MtSK* through several hydrogen bonds with Asp34, Arg58, Glu61, Gly80, and Leu119. The hydrogen bond interaction involving Asp34, Glu61, and Leu119 remained steady during the whole simulation. Based on the RMSD analysis in Figure 4a, it appears that the sinigrin-*MtSK* complex is stable. However, the hydrogen bonds aided by other catalytic residues, as indicated in Hartmann et al. (2006), were shown to be unstable during the simulation. The hydrogen bond formed by Gly80 appears very rarely and has a duration of only 60 nanoseconds, but Arg58 transitions to a

role of facilitating hydrophobic bonding at an early stage of the simulation. Arg136 is infrequently involved in ionic interactions with proteins; perhaps one could argue that it is absent.

Fairly comparable, the interaction between chlorogenic acid and *MtSK* was aided by many hydrogen bonds formed with catalytic residues located inside the active site of *MtSK*, including Asp34, Arg58, Gly80, and Arg136. Gly81, Arg117, and Leu119 were identified as hydrogen bond server according to the MD investigation. However, these interactions, particularly those involving catalytic residues, underwent multiple events, such as alterations in the function of hydrogen interactions or periodic disruptions of their interactions at various points during the simulation. These events may be responsible for the cleavage of the interactions, as depicted in Figure 4a.

Subsequently, we determined the binding energy of protein ligand-complexes using the Poisson-Boltzmann surface area (PBSA) approach as described by Miller et al. (2012) in YASARA Dynamics. The obtained results are visually represented in Figure 4c. The average energy of binding for chlorogenic acid was -158.515 kJ/mol, whereas sinigrin had a binding energy of -56.571 kJ/mol. The native ligand displayed a binding energy of -255.623 kJ/mol. Both chosen ligands exhibited superior binding energy compared to the native ligand, as they had values that were closer to positive. This is due to YASARA's utilization of a specific physics theory, which posits that stronger binding is indicated by more positive energy (Lovering et al. 2005). However, it is important to note that negative energies do not necessarily imply a lack of binding. Therefore, these two ligands have the potential to outcompete the native ligand at the active site.

The calculation of root mean square fluctuation (RMSF) was performed to comprehend the variation of amino acids in protein structure caused by ligands. Figure 4b displays the outcome of the RMSF analysis. This graph aims to compare the fluctuation patterns of individual residues in the protein structure, which were altered by different ligands, including the native ligand (SKM). It is evident that the chlorogenic acid-*MtSK* complex exhibits a greater resemblance to the amino acid fluctuation pattern observed in the SKM-*MtSK* complex, as opposed to the sinigrin-*MtSK* complex. These findings suggest that chlorogenic acid may have a higher potency than sinigrin. This is because the complex formed between chlorogenic acid and *MtSK* produces a comparable impact to the complex formed between *MtSK* and its natural ligand (SKM), in terms of the variations of amino acids.

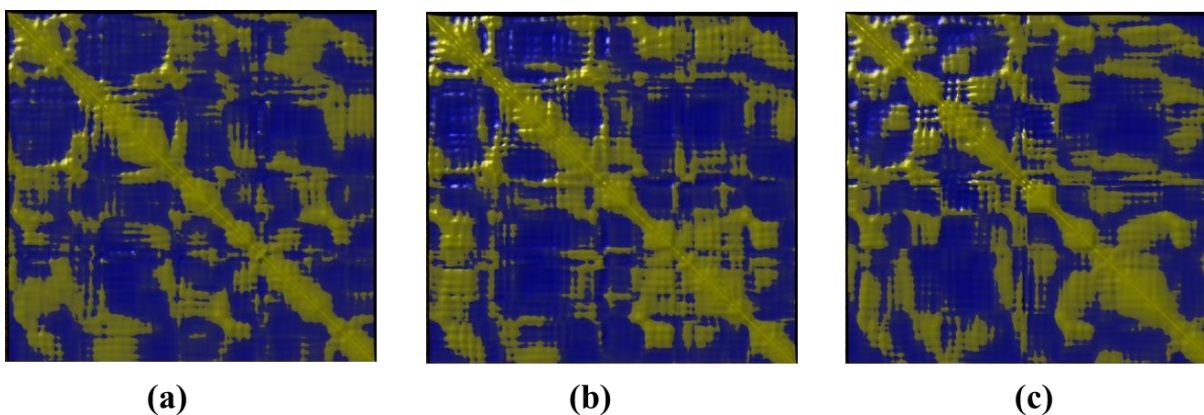


Figure 6. Dynamic cross-correlation matrix (DCCM) of MtSK with selected target complex. (a) Shikimate; (b) Chlorogenic Acid; and (c) Sinigrin

The Dynamic cross-correlation matrix (DCCM) analysis was conducted to evaluate the magnitude and direction of the protein's variations (Yang et al. 2014). Proteins are inherently dynamic, and this characteristic is inherently impacted by the motion of the constituent amino acids. These movements can exhibit correlation or anti-correlation, indicating either the same or opposing direction of movement, respectively. The purpose of this parameter analysis is to assess the impact of specific ligands on protein conformation by analyzing the correlated or uncorrelated motions of residues attached to the ligand site (Doshi et al. 2016), as depicted in Figure 6. The more intense yellow color represents the mobility of residues that are fully correlated (+1) and have the same orientation. Conversely, the presence of the blue color signifies a negative association, in which the residues travel in opposite directions, also known as anti-correlated (-1). Similar to the previous parameter, the study of this parameter was also compared to the SKM-MtSK (Figure 4a) complex as a reference. Both complexes that include specific ligands have an increase in linked movements. Nevertheless, the chlorogenic acid-MtSK complex exhibits a higher degree of similarity to the standard, as evidenced by the correlation and anti-correlation patterns of residue motions, in comparison to the sinigrin-MtSK complex.

ADMET study and druglikeness properties

Two chosen compounds were further analyzed for their ADMET, drug-likeness, and bioavailability properties. The ADMET analysis was conducted using ADMETLab 2.0 (Xiong et al. 2021). Both compounds exhibited favorable distribution characteristics according to PPB, VD,

BBB, and Fu parameters (Figure 7). Nonetheless, the compounds encounter challenges with the excretion of pharmaceutical molecules from the body, as well as concerns linked to permeability through the Caco-2 cell line and medium to high human intestinal absorption (HIA). Sinigrin exhibits a medium-high level of toxicity concerning hepatotoxicity, presenting a significant risk of liver injury, acute oral toxicity, and it may have the potential to cause cancer. Consequently, it is imperative to conduct further evaluative measures to determine the appropriate dosage for treatment or to substitute it with a safer chemical. Meanwhile, chlorogenic acid is devoid of any toxicity concerns.

Compound	Absorption			Distribution				Excretion	Toxicity				
	Caco-2 Permeability	MDCK Permeability	HIA	PPB	VD	BBB Penetration	Fu	CL	H-HT	DILI	AMES Toxicity	Rat Oral Acute Toxicity	Carcinogenicity
Chlorogenic acid	Red	Green	Yellow	Green	Green	Green	Green	Red	Green	Green	Green	Green	Green
Sinigrin	Red	Green	Red	Green	Green	Green	Green	Red	Red	Red	Green	Yellow	Yellow

Figure 7. ADMET analysis of chlorogenic acid and sinigrin. Green color represents desirable properties, and Red color represents non-desirable properties, and Yellow is in between.

Note: Caco-2 Permeability (Permeability); Madin-Darby canine kidney (MDCK); Human Intestinal Absorption (HIA); Human Hepatotoxicity (H-HT); Drug-induced liver injury (DILI); Plasma protein binding (PPB); volume of distribution (Vd); blood-brain barrier permeability (BBB), fraction unbound (Fu).

The druglikeness and bioavailability characteristics were assessed using SwissADME (<http://www.swissadme.ch/>). The druglikeness analysis determines if the compounds comply with Lipinski's Rule of Five (LRO5), allowing for a maximum of one violation. LRO5 is a method designed to predict the drug-like qualities of certain compounds characterized by distinct physicochemical attributes, particularly for oral administration (Lipinski et al. 2001). Both chemicals are suitable for oral administration according to LRO5 data Table 4, while chlorogenic acid has one violation due to exceeding five hydrogen bond donors (six). The bioavailability of the compounds was also assessed, measuring the quantity of unchanged compounds absorbed by the circulatory system. An elevated bioavailability of a specific medicine was essential for achieving optimal concentration in the circulatory system and enhancing pharmacological efficacy (Schelar and Liu 2008; Shanmugavelan and Mohamed Musthafa 2022). The bioavailability score goes from 0 to 1, with a higher number indicating more bioavailability. The table also demonstrates

that chlorogenic acid has a bioavailability score of 0.11, signifying its weak bioavailability, which may necessitate high usual doses to have a specific pharmacological action. Meanwhile, sinigrin possesses a moderate score of 0.55 in this attribute. Certain drug delivery systems may be employed to enhance a drug's bioavailability and achieve the desired pharmacological effect, especially for chlorogenic acid. This analysis also revealed the synthesis complexity level of the chemicals. Both substances exhibit moderate levels with negligible differences, with this parameter ranging from 1 to 10. A lower score indicates greater ease of synthesis for a chemical, and vice versa (Ertl and Schuffenhauer 2009).

Table 4. Druglikeness properties of chlorogenic acid and sinigrin

Compound	Lipins						
	Molecular weight (g/mol) ≤ 500 g/mol	Rule of Five ≤ 1 violation	Bioavailability score	Synthetic accessibility	Moriguchi octanol-water partition coefficient (M log P) ≤ 4.15	Hydrogen bond acceptor (N or O) ≤ 10	Hydrogen bond donors (NH or OH) ≤ 5
Chlorogenic acid	354.31	Yes ; 1 Violation	0.11	4.16	-1.05	9	6
Sinigrin	397.46	Yes ; 0 Violation	0.55	5.35	-2.59	10	4

CONCLUSION

This study identified plant-derived compounds, notably chlorogenic acid and sinigrin, as potential Mycobacterium tuberculosis Shikimate Kinase (*MtSK*) inhibitors. These compounds demonstrated strong binding affinities, stability in the active site, and favorable pharmacokinetic properties through a combination of pharmacophore modeling, molecular docking, molecular dynamics simulations, and ADMET analysis. While chlorogenic acid showed superior stability

and interaction profiles, sinigrin also displayed promising characteristics, though it poses potential toxicity risks that need further investigation. Overall, this research provides a foundation for further developing natural product-based inhibitors targeting MtSK, offering a promising avenue for new anti-tuberculosis therapies. Further experimental studies are needed to validate these findings and optimize these compounds for clinical use.

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