International Journal of Technology

http://ijtech.eng.ui.ac.id

Research Article

Molecular Docking Analysis of Flavonoid from *Strobilanthes crispus* L. as Potential Inhibitors of *Mycobacterium tuberculosis* Targeted Proteins

Rita Arbianti [1], Linatri Purwati Latifah [2], Graciella Sisila [1], Tania Surya Utami [1,3], Ibnu Maulana Hidayatullah [1,3], Fatimah Azizah Riyadi [1,3], Yuswan Muharam [1,*]

[1]*Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, West Java, 16424, Indonesia*
[2]*Yayasan Pendidikan Generasi Nusantara, Jl. Paledang No 02, Katapang, Bandung, West Java, 40971, Indonesia*
[3]*Research Center for Biomass Valorization, Universitas Indonesia, Depok, West Java, 16424, Indonesia*
[*]*Corresponding author: muharam@che.ui.ac.id; Tel.: +62217863516; Fax: +62217863516*

Abstract: Tuberculosis is the 13th leading cause of death worldwide and the major initiator of mortality reported from a single infectious agent (WHO, 2021). The COVID-19 pandemic has increased awareness about the vulnerability of tuberculosis sufferers to the SARS-CoV-2 virus due to possessing compromised immune systems. Since Multidrug Resistant Tuberculosis (MDR-TB) accounts for 78% of the 10 million total cases identified globally, there is an urgent need to develop new anti-tuberculosis drugs. Flavonoid compounds are promising in counteracting antibiotic resistance and enhancing the efficacy of existing anti-tuberculosis treatments. Therefore, this study aimed to conduct a molecular docking analysis of two flavonoid compounds (quercetin and catechin) obtained from *Strobilanthes crispus L.* as potential inhibitors of *Mycobacterium tuberculosis* target proteins using the AutoDock Vina program. The results showed that quercetin was a potent inhibitor of the targeted proteins of *M. tuberculosis*. Furthermore, it produced the highest docking scores of -8.0, -9.2, and -8.0 kcal/mol as well as inhibition constants of 1.345, 0.177, and 1.345 μM for β-ketoacyl-ACP Reductase (PDB ID:1UZN), Enoyl-Acyl Carrier Protein Reductase (PDB ID:2X23), and Protein Kinase G (PDB ID:2PZI), respectively. Based on the obtained molecular docking data, the efficacy of quercetin in inhibiting targeted protein activity should be further tested.

Keywords: Catechin; Molecular docking; *Mycobacterium tuberculosis*; Quercetin; Tuberculosis

1. Introduction

Tuberculosis is the 13th leading cause of death worldwide and the major initiator of mortality reported from a single infectious agent (WHO, 2021). The occurrence of the COVID-19 pandemic has increased awareness about the vulnerability of tuberculosis sufferers to the SARS-CoV-2 virus due to the possession of compromised immune systems and impaired lung conditions. Among the 10 million total tuberculosis cases globally, 78% are classified as Multidrug-Resistant Tuberculosis (MDR-TB). In this context, *Mycobacterium tuberculosis* (*M. tuberculosis*) shows resistance to the two strongest first-line anti-tuberculosis drugs, including rifampicin and isoniazid (Izudi et al., 2020).

The consistent challenges posed by MDR-TB and the side effects of existing anti-tuberculosis drugs drive studies into developing new natural anti-tuberculosis drugs with improved safety profiles (Mazlun et al., 2019). Flavonoid, a class of secondary metabolites, possess potential antibacterial properties that can counteract antibiotic resistance (Gorniak et al., 2018). Previous

https://doi.org/10.14716/ijtech.v16i3.5620
Received April 2022; Revised May 2022; Accepted November 2023

results showed abundant flavonoid presence in *Strobilanthes crispus* L., with a Total Flavonoid Content (TFC) value of 3.3 mg QE/g leaves (Arbianti et al., 2022). Additionally, Ghasemzadeh et al. (2015) identified quercetin and catechin as primary flavonoid compounds in *Strobilanthes crispus* L. Quercetin comprises a flavon structure nC6(ring A)-C3(ring C)–C6(ring B) and is a strong natural antioxidant promoting plant resistance to various biotic and abiotic stressors (Singh et al., 2021). Similarly, catechin is a natural polyphenolic compound belonging to the flavan-3-ols or flavanols group. These two compounds are found to be potential biomarkers for new anti-tuberculosis drugs. Since drug discovery is time-consuming and expensive, in silico computational methods, such as molecular docking, are used to accelerate this process (Sahoo et al., 2022; Jacob et al., 2014).

Considering the discussed perspective, this study aimed to predict and examine the interactions between quercetin and catechin as anti-tuberculosis biomarkers through in silico testing using molecular docking. The AutoDock Vina program was used to perform docking, while the ligand-protein interactions were analyzed with Pymol and Ligplot+. The target proteins of *M. tuberculosis,* including (1) β-ketoacyl-ACP Reductase (MabA) (PDB ID: 1UZN), (2) Enoyl-Acyl Carrier Protein Reductase (inhA) (PDB ID: 2X23), and (3) Protein Kinase G (MtPknG) (PDB ID: 2PZI), were selected for the investigation process. These proteins contribute significantly to the biosynthesis of mycolic acid found in *M. tuberculosis* cell walls and the persistence of tuberculosis pathogens in macrophages (Qasaymeh et al., 2019; Luckner et al., 2010; Cohen-Gonsaud et al., 2002). Mycolic acid protects mycobacteria from the invasion of cationic proteins, lysozymes, and oxygen radicals in phagocytic granules, making it crucial for the virulence and growth of *M. tuberculosis* (Irianti et al., 2016).

MabA is an integral component of the FAS-II enzyme complex, which participates in mycolic acid biosynthesis. Similarly, inhA serves as a trans-2-enoyl-ACP reduction catalyst in FAS-II (Yang and Kong, 2015; Luckner et al., 2010; Cohen-Gonsaud et al., 2002). The protein MtPknG promotes the persistence of tuberculosis pathogens in macrophages by blocking phagosome-lysosome fusion and regulating the signal transduction pathway that controls metabolism (Qasaymeh et al., 2019). In addition to inhibiting mycolic acid biosynthesis, one medium to impede the virulence and metabolism of *M. tuberculosis* is by inactivating MtPknG.

The selection of drug candidates in this study represents the initial phase of exploring new pharmaceutical agents. Applying molecular docking in the selection process is more efficient in terms of time, cost, and test execution compared to in vitro and in vivo methods (Trott and Olson, 2010). Various investigations have used molecular docking to virtually screen the pharmacological potentials of pure compounds, including flavonoid, and identify promising candidates (Sahlan et al., 2023; Sahlan et al., 2020; Ghani et al., 2019).

2. Method

2.1. Hardware

The specifications of hardware used in this study were 8.192 GB RAM, Intel® core ™ i3-6006U CPU @ 2.00GHz (4 CPUs), ~2.0GHz (ASUSTek Computer Inc.), system model X441UA (DirectX 12), and Windows 10 Home Single Language 64-bit operating system (10.0, Build 18362).

2.2. Software

The entire software used included Marvin Sketch (ChemAxon, Budapest) for creating 2D and 3D protein structures, as well as Visual Molecular Dynamics (University of Illinois, Urbana Champaign) for separating the target proteins from the bonded ligand. Additionally, AutoDock Tools version 1.5.6 (The Scripps Research Institute, USA) and AutoDock Vina (The Scripps Research Institute, USA) were used to perform ligand-protein preparation and molecular docking, respectively. PyMOL (www.pymol.org) and LigPlot+ (EMBL-EBI, UK) were applied to visualize the ligand-protein interactions in 3D and 2D formats, respectively.

2.3. Protein Structure Preparation

The preparation of target proteins was conducted based on the process applied by Flamandita et al. (2020). The proteins included MabA, inhA, and MtPknG, which were downloaded from the RSCB Protein Data Bank in .pdb file format.

2.4. Ligand Structure Preparation

Quercetin and catechin test ligands were selected in accordance with the study by Ghasemzadeh et al. (2015) as the predominant flavonoid compounds in *Strobilanthes crispus L.* The potential of these ligands as drug candidates was evaluated with Lipinski's rule of five (RO5). Comparator ligands used were FDA-approved anti-tuberculosis drugs including rifampicin and isoniazid. The 3D structure of the test ligands was created using Marvin Sketch and saved in .pdb format, then loaded into AutoDock Tools 1.5.6. and converted to .pdbqt file format by adding polar hydrogen atoms and specifying the number of torsions.

2.5. Parameter Setting for Docking

Docking parameters were determined by redocking the native ligands of each protein using AutoDock Vina. Subsequently, the resulting conformations were compared with the native crystallographic ligand conformations, expressed as root mean square deviation (RMSD) values. The docking area was centered on the native ligands with a spacing of 1.0 Å to achieve variation in quercetin docking parameters for each target protein.

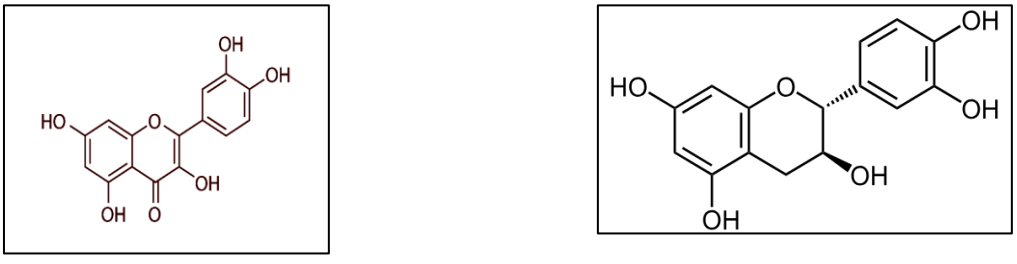
2.6. Molecular Docking Simulation

AutoDock Vina was the main program used for docking quercetin and catechin against the three target proteins, including MabA, inhA, and MtPknG. Docking was performed with a rigid protein and flexible ligand, as it is the most popular method (Meng et al., 2011) and considering computer resources limitation. The scoring function was force field based, where the binding energy is assessed by calculating the sum of the electrostatics and van der Waals interaction (Åqvist et al., 2002). Additionally, the Lamarckian Genetic Algorithm was used to explore the best ligand conformations at the binding sites in the proteins based on ligand flexibility. The maximum number of generations and evaluations was set to 27,000 and 2,500,000, respectively, while other parameters remained at default settings in the docking program. Moreover, visualization of interactions between ligands and proteins followed the procedure described by Nayak et al. (2018).

3. Results and Discussion

3.1. Analysis of Test Ligand Physicochemical Properties

The test ligands were characterized based on Lipinski's rule, often used as a tool in the early stage of drug discovery to evaluate the drug-like properties of chemical compounds (Turner and Agatonovic-Kustin, 2007). Table 1 shows that Quercetin and catechin diagrammatically represented in Figure 1 have molecular weights of 302.238 Da and 290.271 Da, with log*P* values of 2.16 and 1.80, respectively. Both compounds met all Lipinski's criteria, with quercetin featuring 7 hydrogen bond acceptors and 5 donors, while catechin had 6 acceptors and 5 donors. These observations suggest the possession of oral bioavailability and desirable pharmacokinetic properties. Therefore, quercetin and catechin are potential drugs that can serve as anti-tuberculosis biomarkers.



(a) (b)

Figure 1 Chemical structures of (a.) Quarcetin and (b.) Catechin

Table 1 Ligand characteristics evaluation based on Lipinski's rules

No	Test compound	Molecular weight [Da]	Lipophilicity [log*P*]	Total number of hydrogen bond acceptor(s)	Total number of hydrogen bond donor(s)	Total number of unmet prerequisites
1	Quercetin	302.238	2.16	7	5	0
2	Catechin	290.271	1.80	6	5	0

3.2. Parameter Validation for Docking

The native ligands were bound to the complex crystal structure of each target protein, thereby necessitating docking validation through a redocking process. The docking area was located in the test compounds or centered on the ligands using AutoDock Vina. Furthermore, Table 2 shows the docking parameters for the investigated compounds obtained through the docking procedure with the native ligands including Nicotinamide Adenine-Dinucleotide Phosphate (NAP), 5-Hexyl-2-(2-Methylphenoxy)phenol (TCU), and 2-[(Cyclopropylcarbonyl)Amino]-4,5,6,7-Tetrahydro-1-Benzothiopene-3-Carbaxamide (AXX). The redocking results of the three target proteins with the respective native ligands showed RMSD values of < 2 Å. Therefore, the center coordinates and docking area can be used for docking quercetin and catechin against the target proteins.

Table 2 Native ligand docking area parameters from the redocking results

No	Protein	Native ligand	Center coordinates (Center)			Docking area			RMSD (Å)
			X	y	z	x	y	z	
1	MabA [PDB ID: 1UZN]	NAP	5.56	19.684	15.807	28	32	29	0.000
2	inhA [PDB ID: 2X23]	TCU	-20.088	-4.456	-31.407	24	28	24	0.715
3	MtPKnG [PDB ID: 2PZI]	AXX	21.391	-10.215	-4.491	26	25	28	1.112

3.3. Docking Scores and Inhibition Constants

Molecular docking was performed using quercetin and catechin as ligands, while the target proteins included MabA, inhA, and MtPknG. These two compounds were compared to commercial anti-tuberculosis drugs rifampicin and isoniazid (INH). The best binding affinity and conformation of ligands-protein complexes were evaluated with AutoDock Vina through docking scores and inhibition constants. Higher negative docking scores corresponded to lower inhibition constants and higher structural stability of the complexes (Quiroga and Villarreal, 2016). Figure 2 shows the docking scores and inhibition constants of quercetin and catechin against the three target proteins, alongside interactions with native ligands. Quercetin and catechin produced lower docking scores for MabA compared to the native ligand (NADH), while showing binding energies <0, which suggested affinity to the active site. However, both compounds had higher docking scores than the commercial anti-tuberculosis drugs, signifying that more stable conformations were formed with MabA. There was no significant difference in docking scores between quercetin and catechin against MabA, but quercetin had a better inhibition constant, making it a more promising anti-tuberculosis drug candidate. Similar trends were observed for inhA and MtPknG, with quercetin and catechin showing better docking and binding ability toward the active sites than native ligands. The docking score for quercetin across all proteins was the greatest, while the inhibition constants of quercetin and catechin against inhA were smaller compared to other proteins. Based on the results, quercetin is a promising candidate for anti-tuberculosis drug development due to possessing superior binding and inhibition characteristics.

3.4. Molecular Interaction Profile

Quercetin produced the highest docking score and inhibition constant from reacting with MabA, inhA, and MtPknG proteins, leading to the preferred visualization of the molecular interaction including quercetin in this study. To inhibit *M. tuberculosis* growth, quercetin formed hydrogen bonds with amino acid residues Gly^{139}, Arg^{25}, Asn^{88}, Gly^{184}, Gly^{22}, and Ile^{186} in MabA, Asp^{148}, Ser^{94}, Gly^{14}, Ala^{22}, Ser^{20}, and Ile^{194} in inhA, as well as Gly^{237}, Glu^{280}, and Lys^{181} in MtPKnG (Table 3).

Similarities between the interactions of quercetin and comparator ligands including rifampicin and isoniazid with the native ligands MabA, inhA, and MtPknG, are presented in Table 4.

3.5. Molecular interaction visualization

The low molecular weight of both quercetin and catechin adhering to Lipinski's rules (<500 Da) shows that the two compounds can be efficiently absorbed into the bloodstream and transported at a more rapid rate (Smyth et al., 2013; Smyth and Hickey, 2011; Pollastri, 2010). Additionally, the LogP values of 2.16 for quercetin and 1.80 for catechin (<5) signify favorable absorption characteristics in cell membranes, as the majority of biological membranes are lipophilic (Tarcsay and Keserű, 2013; Wen and Park, 2010). Molecular docking simulation requires considering the stability of ligand-target protein complexes formed, which is influenced by hydrogen bonding between the two entities. Therefore, the number of hydrogen bond donors and acceptors affects the stability of compounds to penetrate membrane layers (Lipinski et al., 2001). Due to the compliance of quercetin and catechin with Lipinski's rules, both compounds have good bioavailability and permeability in the body.

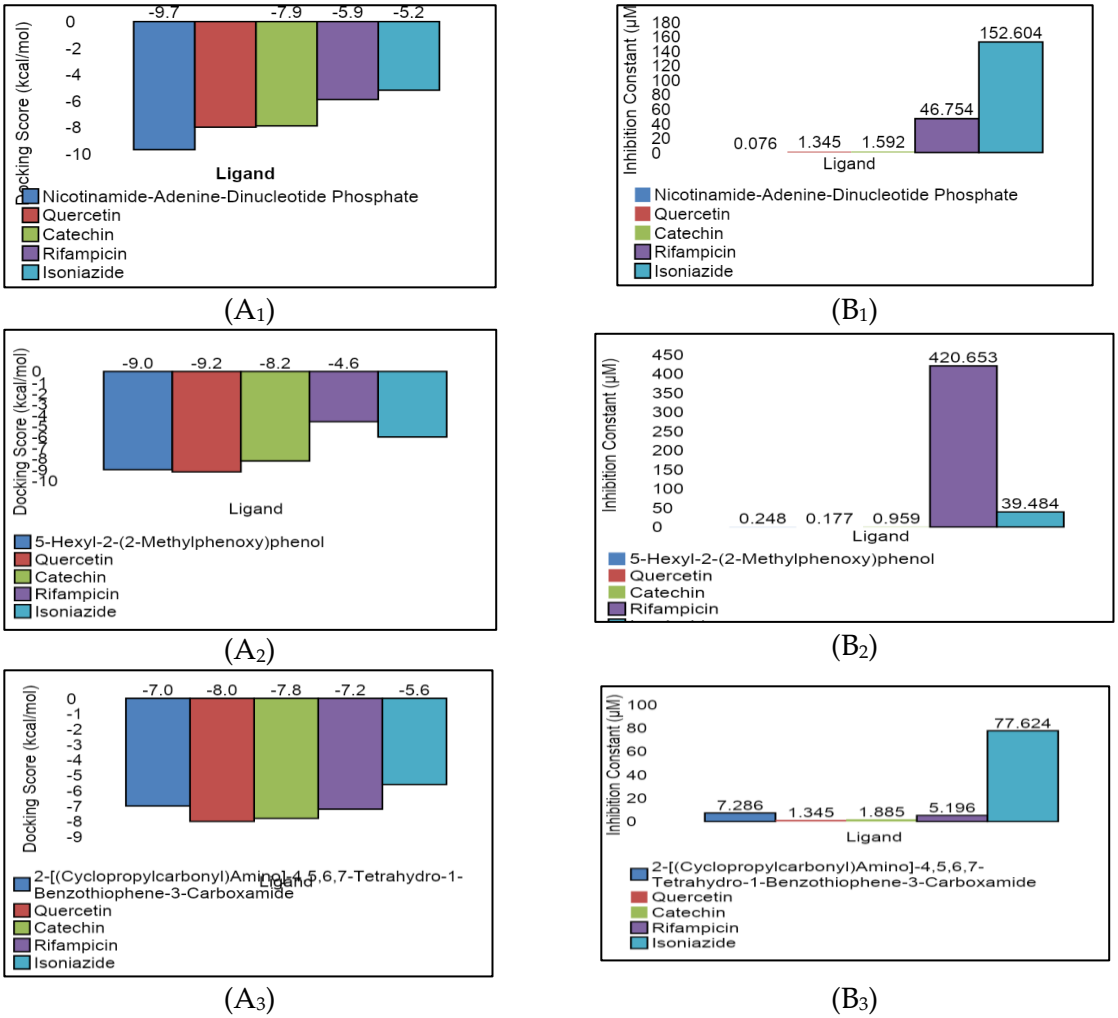


Figure 2 Docking score (A1; A2; A3) and inhibition constant (B1; B2; B3) of each ligand against MabA, inhA, and MtPknG

MabA, inhA, and MtPknG target proteins used in this study have native ligands bound to the complex crystal structures. The RMSD values of <2 for the generated redocking results signify minimal deviation between the native crystallographic ligands and the redocked conformations, ensuring more accuracy in interaction predictions (Listyani et al., 2019). This enables the use of native ligand active sites on the target proteins for quercetin and catechin docking processes (Table 2). The results show the superior potential of quercetin as an anti-tuberculosis biomarker, evidenced by the docking score and lowest inhibition constant. Quercetin also outperforms catechin, rifampicin, and isoniazid, as well as the native ligands in inhibiting target proteins of *M. tuberculosis,* such as inhA and MtPKnG. Various in silico studies have previously explored the potential of quercetin as a growth inhibitor of *M. tuberculosis*. For instance, Herli et al. (2016) reported a score of -6.8 kcal/mol from quercetin molecular docking against *M. tuberculosis* RNA Polymerase. This was consistent with the results by Lisnyak and Martynov (2019) that quercetin inhibited *M. tuberculosis* Urease (MTU) with a docking score of -8.7 kcal/mol and inhibition constant of 0.4 µM.

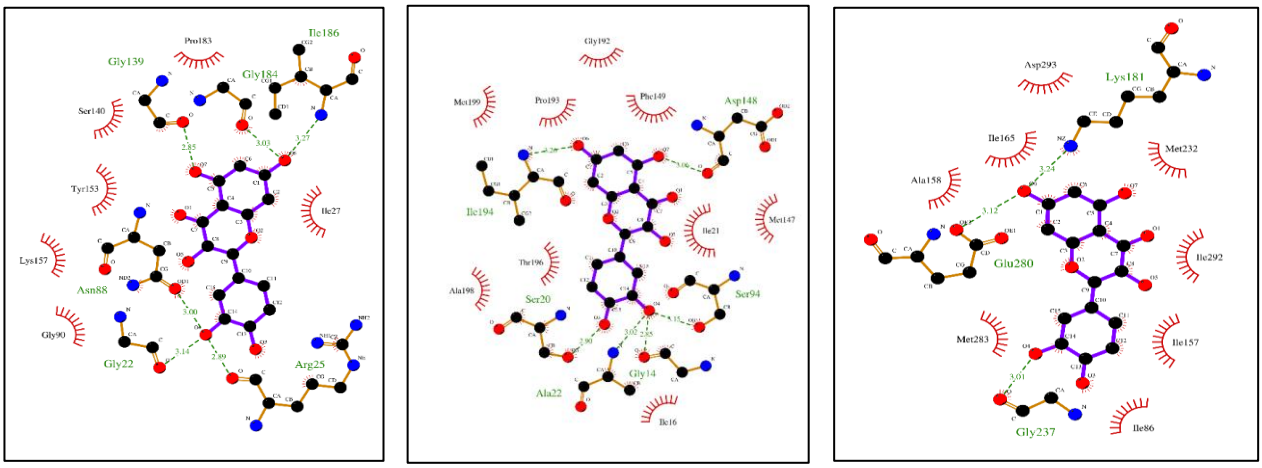
Table 3 Molecular interactions between quercetin and target proteins

Protein target	Number of interaction(s)	Hydrogen bond distance (Å)	Amino acid residue	Hydrophobic interaction(s)
MabA	12	2.85	Gly^{139}	Gly^{90}, Lys^{157}, Tyr^{153}, Ser^{140}, Pro^{183}, Ile^{27}
		2.89	Arg^{25}	
		3.00	Asn^{88}	
		3.03	Gly^{184}	
		3.14	Gly^{22}	
		3.27	Ile^{186}	
inhA	15	2.85	Gly^{14}	Pro^{193}, Met^{199}, Gly^{192}, Phe^{149}, Ile^{21}, Thr^{196}, Ala^{198}, Met^{147}, Ile^{16}
		2.90	Ser^{20}	
		3.02	Ala^{22}	
		3.06	Asp^{148}	
		3.15	Ser^{94}	
		3.26	Ile^{194}	
MtPknG	11	3.01	Gly^{237}	Ile^{86}, Ile^{157}, Ile^{292}, Met^{232}, Asp^{293}, Ile^{165}, Ala^{158}, Met^{283}
		3.12	Glu^{280}	
		3.24	Lys^{181}	

Table 4 Comparison of quercetin, rifampicin, and isoniazid interactions with MabA, inhA, and MtPknG

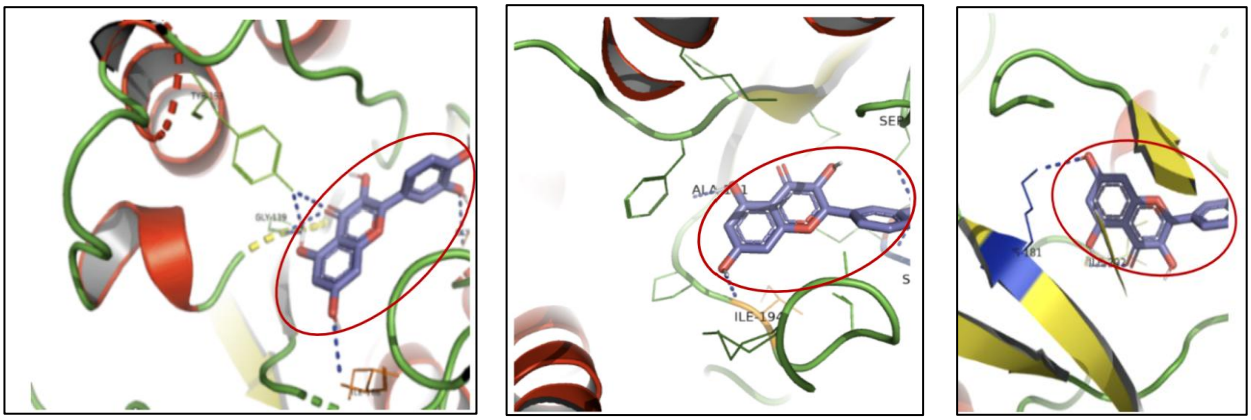
Protein Target	Interaction Similarity			Total Similarity (%)		
	Rifampicin	Isoniazid	Native Ligand	Rifampicin	Isoniazid	Native Ligand
MabA	Arg^{25}	Arg^{25}, Asn^{88}, Gly^{22}, Ile^{27}, Gly^{90}	Asn^{88}, Gly^{22}, Gly^{90}, Ile^{27}, Arg^{25}	8.33	41.67	41.67
inhA	-	-	Met^{199}, Pro^{193}, Ala^{198}, and Phe^{149}	-	-	33.33
MtPknG	Ile^{157}, Met^{283}, Ala^{158}, Ile^{86}	Asp^{293}, Lys^{181}, Glu^{280}, Ile^{165}, Ala^{158}	Ala^{158}, Ile^{165}, Ile^{292}, Ile^{157}, Gly^{237}, Met^{283}	36.3	45.5	54.5

The 2D and 3D visualization of interactions occurring between quercetin and MabA, inhA, and MtPKnG proteins are shown in Figures 3 and 4, respectively. According to Figures 3a/4a, hydrogen bonds were formed with Gly^{139}, Arg^{25}, Asn^{88}, Gly^{184}, and Gly^{22} residues in MabA due to the interactions between the hydroxyl group (-OH) in quercetin with the O atom in the C=O group main chain of the amino acid residues serving as a proton acceptor (OH… O). Hydrogen bonds between quercetin and Ile186 resulted from the interaction of the N atom of the R-NH_2 group in Ile^{186} as a proton donor with the O atom in quercetin. Additionally, quercetin formed hydrophobic interactions with amino acid residues Gly^{90}, Lys^{157}, Tyr^{153}, Ser^{140}, Pro^{183}, and Ile^{27} in MabA.



(a) (b) (c)

Figure 3 2D visualization of Quercetin interactions with MabA (a); inhA (b); and MtPknG (c) (: Nitrogen Atom, : Oxygen Atom, : Hydrophobic Interaction, - - : Hydrogen Bond)



(a) (b) (c)

Figure 4 3D visualization of Quercetin interactions with MabA (a); inhA (b); and MtPknG (c)

Based on the information from the study of Shilpi et al. (2015), Lys^{157}, Tyr^{153}, and Ser^{140} are regarded as catalytic triads, which refer to a set of amino acids working jointly on the active sites of MabA. Tyr^{153} has a major role in acid-base catalysis, while Asn^{140} is capable of eliminating protein activity, and Gly^{90} participates in the complexation of MabA with the respective native ligands.

Other hydrogen bonds occur between quercetin and Asp^{148}, Ser^{94}, Gly^{14}, Ala^{22}, Ser^{20}, and Ile^{194} residues in inhA (Figure 3b/4b). The bonds formed with Asp^{148}, Ser^{94}, Gly^{14}, and Ser^{20} resulted from the interactions of the H atom in the -OH group of quercetin as a proton donor with the O atom in the C = O group of the residues as a proton acceptor. Apart from acting as a proton donor molecule, quercetin attached to inhA acted as an acceptor, similar to Ala^{22} and Ile^{194}. The N atom from the R-NH_2 group of these two residues donated proton to the O atom of quercetin (N-H…O). The amino

acid residues in inhA that formed hydrophobic interactions with quercetin included Pro^{193}, Met^{199}, Gly^{192}, Phe^{149}, Ile^{21}, Thr^{196}, Ala^{198}, Met^{147}, and Ile^{16}.

Hydrophobic interactions played a crucial role in stabilizing the test compounds bound to the target proteins by avoiding a liquid environment in the globular structure of the proteins to minimize the interactions of non-polar residues with water (Camilloni et al., 2016). Apart from MabA and inhA, quercetin is capable of inhibiting *M. tuberculosis* virulence in the body through hydrogen bonding with Gly^{237}, Glu^{280}, and Lys^{181} residues in MtPknG (Figure 3c/4c). The amino acid residues directly contribute to the activities of MtPknG in maintaining the persistence of tuberculosis pathogens in macrophages.

Hydrogen bonds formed with Gly^{237} resulted from the interactions between the O atom in the C=O group which received a proton from -OH in quercetin. Additionally, hydrogen bonding between Glu^{280} in MtPknG and quercetin occurred because the O atom in quercetin provided a proton to Glu^{280}. The bonds formed between quercetin and Gly^{237} and Glu^{280} residues were stronger than in Lys^{181}, perhaps due to the high reactivity of the hydroxyl groups on this flavonoid as hydrogen donors (Alfaridz and Amalia, 2018; Chirumbolo, 2010). The hydrogen bond formed with Lys^{181} in MtPknG was weak because -OH in quercetin acted as an acceptor of proton donated from the R-NH_2 group in Lys^{181} (N-H… O).

The overall in silico test in this study showed that quercetin had a similar performance with rifampicin and isoniazid in inhibiting the metabolism and virulence of *M. tuberculosis* in the body. This was indicated by various hydrophobic interactions and hydrogen bonds formed between quercetin and amino acid residues in the target proteins of *M. tuberculosis*. Based on Table 3, quercetin was more identical to the commercial anti-tuberculosis drug isoniazid compared to rifampicin by producing a total similarity of 41.67% on MabA and 45.5% on MtPknG proteins. Furthermore, MtPknG had the highest similarity percentage of 54.4% in terms of the interactions formed between quercetin and native ligands, compared to MabA and inhA.

4. Conclusions

In conclusion, the results of in silico testing conducted using AutoDock Vina showed that quercetin obtained from *Strobilanthes crispus* L. had potential as an antimycobacterial agent for *M. tuberculosis*. Additionally, quercetin manifested inhibitory activities against MabA, inhA, and MtPknG proteins known to be extremely crucial in the virulence and survival of *M. tuberculosis*. The general results significantly contributed to the field of alternative herbal-based treatments for tuberculosis. Therefore, in vitro and in vivo experiments should be performed to assess the efficacy of quercetin in inhibiting *M. tuberculosis* target proteins.

Acknowledgements

The authors are grateful to the University of Indonesia for the funding assistance offered through the PUTI (Publikasi Terindeks Internasional) Doctoral Research Grant 2020 (Grant Number: NKB-666/UN2.RST/HKP.05.00/2020) and the Department of Chemical Engineering for the valuable support provided.

Author Contributions

R.A., T.S.U., and Y.M. designed the study. L.P.L., G.S., and R.A. conducted the experiments. L.P.L., R.A., T.S.U., and Y.M. prepared the initial manuscript. L.P.L., R.A., T.S.U., Y.M., F.A.R., and I.M.H. analysed the data and contributed to its interpretation. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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