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Natural flavonoids in *Delonix regia* leaf as an antimycobacterial agent: An *in silico* study

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Abstract: Multi-drug resistant (MDR) and extensively-drug resistant (XDR) as results of continuous use of antibiotics encourage the development of new antimycobacterial drugs. In this study, 13 flavonoid compounds from the flamboyant leaf plant were studied for their inhibitory properties of *Mt*KasA, *Mt*DprE and *Mt*Pank which are significant enzymes in *Mycobacterium tuberculosis*, as well as for their molecular docking, molecular dynamics and prediction of ADMET-drug likeness. The results of molecular docking studies revealed that compound F13 (apigenin) was the most potent compound because it was able to bind the most amino acids as indicated by the native ligand of each enzyme. Molecular dynamics studies showed that compound F13 forms a stable complex with *Mt*KasA. The results of the ADMET-drug likeness analysis concluded that compound F13 was the most promising compound. Overall, compound F13 has the potential to be used as a treatment therapy against *M. tuberculosis.*

Keywords: tuberculosis; molecular docking; *Mt*KasA; *Mt*DprE; *Mt*Pank.

INTRODUCTION

Tuberculosis (TB) is a deadly disease caused by *Mycobacterium tuberculosis*. Many parties have and are paying special attention to the handling of tuberculosis. WHO, in the Global Tuberculosis Report 2021, reported that the pandemic of COVID-19 has a negative impact on efforts to treat tuberculosis. Limited access to diagnosis and treatment of tuberculosis has increased the number of tuberculosis deaths by 1.2 million since 2019.[1] WHO also noted four countries contributed the most prominent TB cases, namely India (41 %), Indonesia (14 %), the Philippines (12 %) and China (8 %). Another report noted that cases of drug-

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-resistant TB against first-line drug usage for TB therapy have increased by 15 %.[1] This is primarily due to the prolonged treatment of TB, as well as the use of antibiotic combinations with side effects that result in the development of multi-drug resistant (MDR) and extensively drug-resistant (XDR).[2] As a result of all these reports, tuberculosis treatment needs urgent attention as well as appropriate and quick solutions.

There are still many drug discovery studies underway to overcome the problem of drug resistance, including tuberculosis.[3] Molecular docking is a computational method for new drug discovery based on structure-based design. Application of molecular docking methods includes virtual screening, prediction of adverse drug reactions, drug repositioning, and drug mechanism of action against the targeted protein.[4] Therefore, molecular docking is an excellent and powerful method for predicting drug activity against specific proteins. Several targeted proteins to predict drug activity against the growth of *M. tuberculosis* are *Mt*KasA, *Mt*DprE1 and *Mt*Pank.[5] *Mt*KasA and *Mt*DprE1 are necessary enzymes used for cell wall biosynthesis. *Mt*KasA is responsible for elongating the fatty acid carbon chain in the FAS-II pathway and provides a precursor for mycolic acid biosynthesis.[6] *Mt*DprE1 performs the epimerization reaction of decaprenyl phosphoryl-*β*-D-ribose (DPR) to decaprenyl phosphoryl-*β*-D-arabinofuranose (DPA) in the periplasmic space of the cell membrane. DPA is used for the biogenesis of arabinogalactan and lipoarabinomannan.[7,8] *Mt*Pank is also an essential drug target to overcome drug-resistant TB. *Mt*Pank enzyme plays a role in the growth of *M. tuberculosis* through the biosynthesis of coenzyme-A (CoA).[9]

Various studies have been conducted to study the plant's active compounds and their biological activity against *M. tuberculosis*.[10–13] The content of secondary metabolites in plants significantly contributes to certain biological activities and is always accompanied by low side effects.[14] Among the secondary metabolites, flavonoids have been reported to have inhibitory activity against the growth of *M. tuberculosis*.[15–17] Chemically, flavonoids have two benzene rings, A and B, which are connected by a pyran heterocyclic ring (C ring). Flavonoid groups include flavonols, flavanones, flavones, isoflavones, flavanols and anthocyanins.[18]

Flamboyant (*Delonix regia*) is a plant that comes from the *Caesalpiniaceae* family and is widely found in various regions of Indonesia. Previous studies reported that the isolation of flamboyant leaves with ethanol as a solvent produced 13 flavonoid compounds.[19] The presence of flavonoids led to the investigation of their ability to inhibit 3 key enzymes in the growth of *M. tuberculosis*. Therefore, this study aimed to identify the potential of flavonoid compounds contained in flamboyant leaves as antimycobacterial agents using an *in silico* study approach. As a result, the potential compounds obtained could be used as reference compounds for laboratory testing.

EXPERIMENTAL

Materials

As test ligands, natural flavonoids were obtained from previous studies investigating the HPLC profiles of *Delonix regia* leaves extract.[19] The proteins analyzed in this study include *Mt*KasA, *Mt*DprE1 and *Mt*Pank taken from the RSCB database with PDB ID were 2WGE, 4FF6 and 4BFT, respectively. The software used for molecular docking was AutoDock Vina; the structures of ligand compounds were drawn with ChemOffice Professional 16 and Chem3D Professional 16, and structure optimization was performed using Gaussian 09. Molecular docking results were visualized in Discovery Studio client 2021.

Validation of docking protocol

Each receptor protein was prepared by separating the native ligand from the protein using AutoDock Tool (ADT) 1.5.6. Missing atoms of the receptor protein were checked and corrected through ADT. Furthermore, the water molecule on the receptor protein was cleaned and the protein was added with Kollman charge and then stored in the pdbqt extension format. The separated native ligands were prepared by adding hydrogen atoms and Gasteiger charge.

Prepared proteins and native ligands were then docked by following several parameters. The molecular docking for *Mt*KasA protein used a grid box dimension of 25×25×25 Å^3 with cartesian coordinates *x*, *y* and *z* of 37.883, 0.813 and –5.898, respectively. *Mt*DprE1 protein used a grid box size of 12×12×12 Å^3 with cartesian coordinates *x*, *y* and *z* of 14.697, –21.165 and 36.955, respectively, and for *Mt*Pank protein, it was 14×14×14 Å^3 with cartesian coordinates *x*, *y* and *z* of –19.242, –10.217 and 12.648, respectively. An exhaustiveness of 16 was applied to the protocol docking validation process. In order to dock all proteins, the spacing value of 1 Å was used. *RMSD* value was determined using Pymol. The docking protocol was used to dock the tested compound if it had an *RMSD* value below 2 Å.[20]

Preparation of natural flavonoid as tested ligand

The structure of natural flavonoids from *D. regia* leaf extract was taken from the PubChem database. Compounds that were not found in the database were drawn with ChemDraw Professional 16, as shown in Table I. Furthermore, all compounds were converted to 3-dimensional structure and geometric optimization using Gaussian 09 with the DFT method with a base set B3LYP 6-31G.[21] Furthermore, the test compound was prepared using ADT, which involved the addition of hydrogen atoms, merging non-polar hydrogen atoms and adding Gasteiger charge. All compounds that had been prepared were then stored in a pdbqt format file.

Antimycobacterial profile prediction

The antimycobacterial properties of 13 flavonoids contained in flamboyant leaf extract were predicted. Each SMILES code of the flavonoid compound was inputted into PASS online (http://www.way2drug.com/passonline/predict.php). Activity predicted values (*Pa*) and inactive predictions were recorded, tabulated and presented in graphical form.

Molecular docking

Natural flavonoids that had been optimized were docked to the three receptor proteins. Docking was conducted using AutoDock Vina following a validated docking protocol. The docking results generated binding energy as a value that indicated the binding energy between the ligands and the receptor protein. The 2D and 3D interactions of the tested compound with key amino acid residues of the receptor protein were visualized using Discovery Studio client 2021.

ADMET-drug-likeness analysis

Pharmacokinetic properties of natural flavonoid compounds, including adsorption, distribution, metabolism, excretion, and toxicity, were predicted using the pkCSM tool.[22] The drug-likeness properties were studied using the MolSoft tool (https://www.molsoft.com/publications.html).

Molecular dynamic simulation

The lead compounds obtained from molecular docking simulations were then studied for the stability of their interactions with amino acid receptors, in this case *Mt*KasA, *Mt*DprE1 and *Mt*Pank. This was done through molecular dynamic simulations. Simulations used CABS Flex 2.0 server (http://biocomp.chem.uw.edu.pl/CABSflex2/index). The simulation setting used a number of cycles and 50 trajectory frames. Furthermore, the temperature of the simulation and the global weight were respectively 1.4 and 1.0.

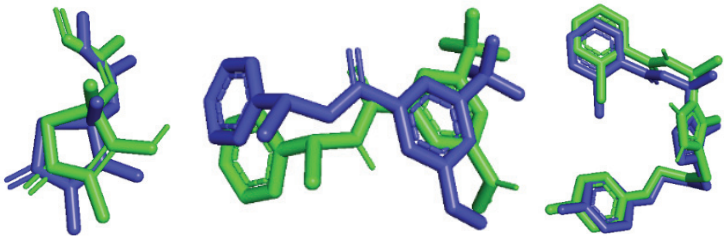
RESULTS AND DISCUSSION

Validation of docking protocol

The molecular docking method is a powerful in silico method for predicting the interaction between ligands and receptors.[23] To study the antimycobacterial properties of natural flavonoids in the ethanolic extract of *Delonix regia* leaves, three significant proteins were used to inhibit *Mycobacterium tuberculosis* activities. These proteins were *Mt*KasA, *Mt*DprE1 and *Mt*Pank. Docking protocol validation was performed before molecular docking of the test compounds was carried out on the three proteins. As shown in Fig. 1, the native ligands are superimposed and include *Mt*KasA complexes with thiolactomycin (TLM), *Mt*DprE1 complexes with 3-(hydroxyamino)-*N*-[(1*R*)-1-phenylethyl]-5-(trifluoromethyl)-benzamide (0T4) and *Mt*Pank with 2-chloro-*N*-[1-(5-[[2-(4-fluorophenoxy)sulfanyl]-4-methyl-4*H*-1,2,4-triazo-3-yl]benzamide (ZVT). Validation results in the form of *RMSD* values for *Mt*KasA, *Mt*DprE1 and *Mt*Pank were 0.828, 1.512 and 0.689 Å, respectively.

Antimycobacterial profile prediction

Prediction of the antimycobacterial profile of flavonoid compounds was carried out using the PASS server and gave results as shown in Fig. 2. The results of



(A) (B) (C)

Fig. 1. Superimpose re-docking ligand (green) into native ligand (blue) of protein receptor: A) complex of *Mt*KasA (id pdb: 2wge) – TLM; B) complex of *Mt*DprE1 (id pdb: 4ff6) – 0T4; C) complex of *Mt*Pank (id pdb: 4bft) – ZVT.

the analysis from the PASS server showed that all compounds had a *Pa* value >0.3, however the value was still <0.7 except for compound F5 (0.276). This means that the compound was predicted to have moderate antimycobacterial potential in the laboratory.[24] Furthermore, this compound could be further confirmed through molecular docking.

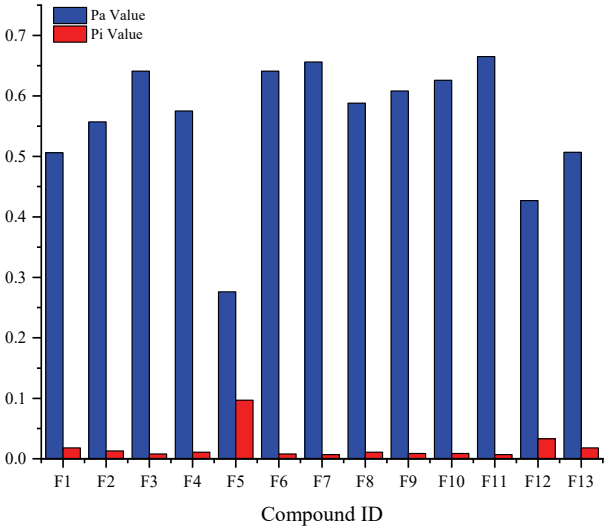


Fig. 2. Graph of antimycobacterial profile prediction by flavonoid compounds from flamboyant leaves.

Molecular docking

The chemical structure of flavonoid compounds contained in flamboyant leaf extract that were used as tested ligands, is shown in Table I. In this study, molecular docking of flavonoids in flamboyant plants was conducted to determine potential compounds for antituberculosis drugs. The binding energy and chemical interactions of each flavonoid with the targeted protein were studied and compared to natural inhibitors of each enzyme.

Molecular docking of the *Mt*KasA enzyme was carried out on 13 flavonoids contained in flamboyant leaves and the results are shown in Table II. For *Mt*KasA molecular docking, TLM which acted as a natural inhibitor, produced a binding energy value of –32.22 kJ/mol, while some other test compounds also demonstrated satisfactory inhibitory activity compared to natural inhibitors. Compound F6 had the highest binding energy (–38.91 kJ/mol), followed by the compound F5 with a binding energy value of –36.40 kJ/mol, while compounds F3, F12 and F13 showed the same binding energy value of –35.95 kJ/mol.

An investigation of the chemical interaction of TLM in the *Mt*KasA enzyme binding pocket has been carried out (Table S-I of the Supplementary material to

TABLE I. Natural flavonoid compounds from Flamboyant leaves (*D. regia*)

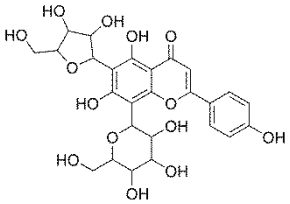
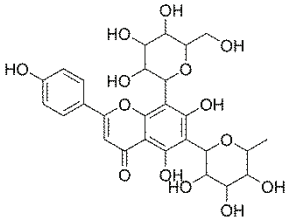
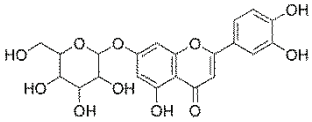
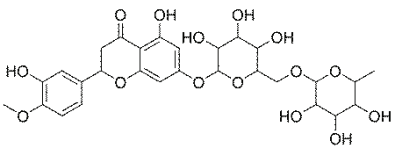
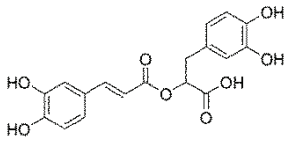
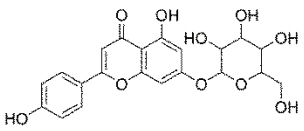
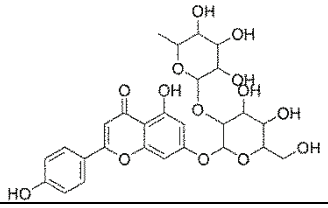
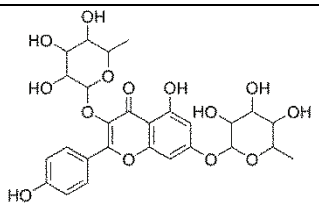
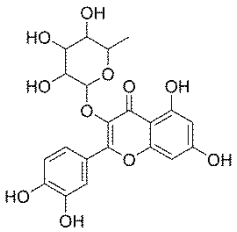
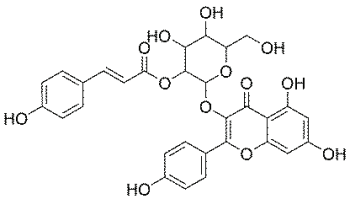
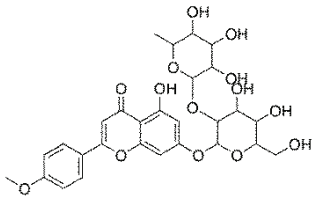
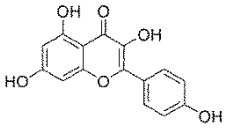
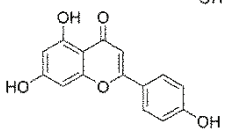
Comp. ID	Chemical structure	PubChem ID	IUPAC name (PubChem generated)
F1		137832345	6-[3,4-Dihydroxy-5-(hydroxymethyl)oxolan-2-yl]--5,7-dihydroxy-2-(4-hydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-chromen-4-one
F2		74977441	5,7-Dihydroxy-2-(4-hydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-6--(3,4,5-trihydroxy-6-methyloxan-2-yl)-chromen-4-one
F3		5291488	2-(3,4-Dihydroxyphenyl)--5-hydroxy-7-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-oxychromen-4-one
F4		3594	5-Hydroxy-2-(3-hydroxy--4-methoxyphenyl)-7--[3,4,5-trihydroxy-6--[(3,4,5-trihydroxy-6-methyloxan-2-yl)oxymethyl]oxan-2-yl]oxy--2,3-dihydrochromen-4--one
F5		5315615	3-(3,4-Dihydroxyphenyl)--2-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid
F6		5385553	5-Hydroxy-2-(4-hydroxyphenyl)-7-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-oxychromen-4-one
F7		5459217	7-[4,5-Dihydroxy-6-(hydroxymethyl)-3-(3,4,5-trihydroxy-6-methyloxan-2--yl)oxyoxan-2-yl]oxy-5--hydroxy-2-(4-hydroxyphenyl)chromen-4-one

TABLE I. Continued

Comp. ID	Chemical structure	PubChem ID	IUPAC name (PubChem generated)
F8		12305415	5-Hydroxy-2-(4-hydroxyphenyl)-3,7-bis[(3,4,5--trihydroxy-6-methyloxan-2-yl)oxy]chromen--4-one
F9		5353915	2-(3,4-Dihydroxyphenyl)--5,7-dihydroxy-3-(3,4,5--trihydroxy-6-methyloxan-2-yl)oxychromen-4--one
F10		131752460	[2-[5,7-Dihydroxy-2-(4--hydroxyphenyl)-4-oxochromen-3-yl]oxy-4,5--dihydroxy-6-(hydroxymethyl)oxan-3-yl] (*E*)-3--(4-hydroxyphenyl)prop--2-enoate
F11		15559329	7-[4,5-Dihydroxy-6-(hydroxymethyl)-3-(3,4,5-trihydroxy-6-methyloxan-2--yl)oxyoxan-2-yl]oxy-5--hydroxy-2-(4-methoxyphenyl)chromen-4-one
F12		5280863	3,5,7-Trihydroxy-2-(4--hydroxyphenyl)chromen--4-one
F13		5280443	5,7-Dihydroxy-2-(4-hydroxyphenyl)chromen-4--one

this paper). The oxygen atom of the thiolactone ring is linked to Cys171 by hydrogen bonds. The binding of His311 was formed by the pi-sulfur bond on the S atom of the thiolactone ring. The bond stabilization in the enzyme *Mt*KasA binding pocket was facilitated by hydrophobic interactions with amino acids Phe237, Phe404 and Pro280. This fact was in accordance with the results of a study conducted and reported by Luckner *et al*. that the catalytic triad Cys171-His311-

-His345 was the active site of the *Mt*KasA binding pocket (Fig. 3).[25] Compounds F5 and F6 showed hydrogen bonds to the His311 amino acid. Hydrogen bonds also supported the stabilization of compound F6 in interacting with Pro280. Compound F5 has slightly lower binding energy, whereas binding of the compound to Pro280 involves hydrophobic interactions. The loss of hydrogen bonds in the catalytic triad was suspected as a reason for the lower binding energy of compounds F3, F12 and F13. Additionally, compounds F3, F12 and F13 involving hydrophobic interactions also bind to essential amino acids like Cys171, Pro280 and Phe404. This evidence was an indication that compounds F3, F12 and F13 also interact at the *Mt*KasA enzyme active site.

TABLE II. Binding energy (kJ/mol) of natural flavonoid compound from *Delonix regia* leaf

ID compound	Name	Enzyme		
		*Mt*KasA	*Mt*DprE1	*Mt*Pank
TLM	Thiolactomycin	–32.22	–	–
0T4	3-(Hydroxyamino)-*N*-[(1*R*)-1-phenylethyl]-5--(trifluoromethyl)benzamide	–	–37.66	–
ZVT	2-Chloro-*N*-[1-(5-{[2-(4-fluorophenoxy)-ethyl]sulfanyl}-4-methyl-4*H*-1,2,4-triazol-3--yl)ethyl]benzamide	–	–	–39.33
	Flavonoid compounds			
F1	Apigenin-6-arabinose-8-galactose	–28.03	–33.47	–36.82
F2	Apigenin-6-rhamnose-8-glucosa	–28.87	–33.47	–34.31
F3	Luteolin-7-glucose	–35.98	–35.56	–36.82
F4	Hesperidin	–35.15	–34.31	–35.15
F5	Rosmarinate	–36.40	–33.47	–30.96
F6	Apigenin-7-*O*-glucose	–38.91	–37.66	–39.33
F7	Apigenin-7-*O*-neohespiroside	–31.38	–35.98	–32.64
F8	Lespedin	–30.96	–35.98	–31.38
F9	Quercetin-3-*O*-rhamnoside	–24.69	–33.47	–34.73
F10	2"-*O*-Trans-*P*-coumaroylastragalin	–30.54	–39.75	–39.33
F11	Fortunellin	–31.80	–34.31	–37.66
F12	Kaempferol	–35.98	–33.89	–34.31
F13	Apigenin	–35.98	–34.31	–33.05

Inhibition of the tested compounds against the *Mt*DprE1 enzyme was also studied. Molecular docking revealed that all analyzed compounds were potent inhibitors of the *Mt*DprE1 enzyme with binding energies ranging from –33.89 to –39.75 kJ/mol. Compound F10 was the tested compound with the largest binding energy of –39.75 kJ/mol, while the compound F6 produced the same binding energy as the native ligand (0T4) of –37.66 kJ/mol. In this study, we have also found that the core structure of flavonoid compounds in apigenin and kaempferol had excellent binding ability. Compounds F7 and F13 with an apigenin core structure had binding energies of –35.98 and –34.31 kJ/mol, respectively. Com-

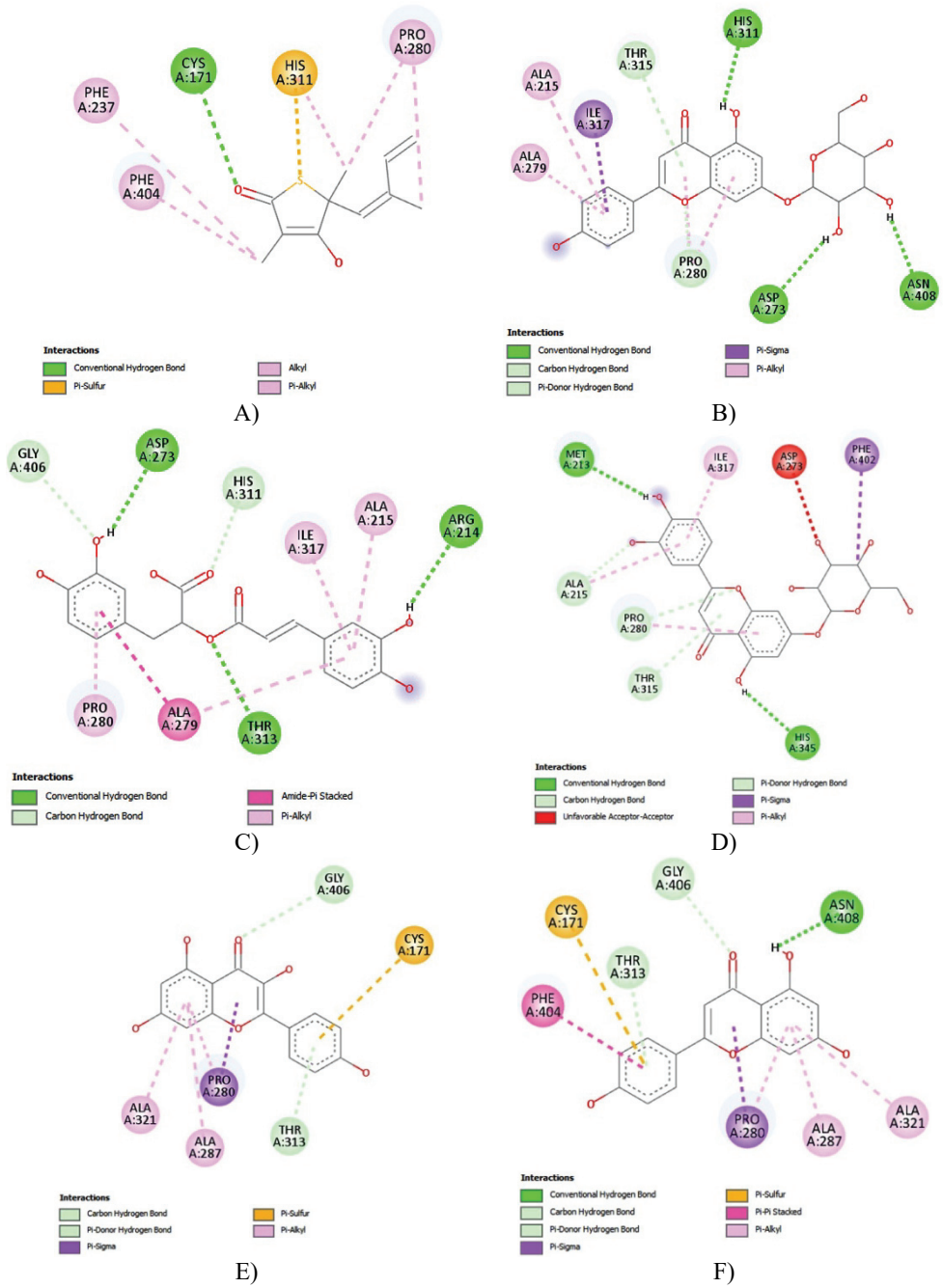


Fig. 3. Chemical interaction of *Mt*KasA enzyme with: A) TLM; B) compound F6; C) compound F5; D) compound F3; E) compound F12; F) compound F13.

pounds F8 and F12, which had a kaempferol core structure, gave bond affinities of –35.98 and –33.89 kJ/mol, respectively.

The chemical interaction visualization of the tested compounds against the *Mt*DprE1 enzyme provided data to support data binding energy. 0T4 compound as a native ligand bound several key amino acids (Table S-II of the Supplementary material). Cys387 amino acid is found in the substrate-binding domain of *Mt*DprE1 enzyme.[26] In addition, several amino acids, such as Lys134, Gly117 and Gly113, which are located in the FAD-interacting domain,[7] were also seen in Fig. 4. Compound F10 was able to bind the amino acid Cys387 through hydrogen bonding. Lys134 and Gly117 amino acids were also bonded *via* hydrogen bonding with the compound F10. For the compound F7, adherence to Cys387 and Lys134 amino acids involved hydrophobic interactions, and the compound F13 only attached Lys134 *via* hydrogen bonding. In this case, the hydroxyl group of the rhamnoside substituent is used to bond the compound F8 to Cys387 essential amino acid. The weak binding affinity of the compound F12 was due to hydrophobic interactions attached to Cys387 amino acid, according to binding energy data.

Molecular docking studies of the tested compounds were also carried out on pantothenate kinase (*Mt*Pank). Binding energy data showed that all the tested compounds had excellent affinity potential for the *Mt*Pank enzyme, ranging from –30.96 to –39.33 kJ/mol. This study used the native ligand ZVT as a comparison ligand. The molecular docking results showed that flavonoid core structures in apigenin and kaempferol gave the most effective binding affinity, as could be seen for compounds F6 and F10 with a binding energy value of –39.33 kJ/mol. It was also revealed that compounds derived from the apigenin core structure, such as those in compounds F1 and F2 resulted in slightly lower binding energy in molecular docking results. With the *Mt*Pank, the compound F11 also produced a fairly significant binding affinity of –37.66 kJ/mol compared to *Mt*KasA and *Mt*DprE1 enzymes. In accordance with the binding energy value, the compound F11 did not have the ability to bond to *Mt*KasA or *Mt*DprE1.

It was interesting to discuss the chemical interactions between all tested compounds and the *Mt*Pank enzyme. ZVT compounds as native ligands showed interactions in the *Mt*Pank binding pocket (Table S-III of the Supplementary material). The triazole ring of ZVT compound formed hydrogen bonds with Tyr235 and Asn277 with bond lengths of 1.94 and 2.46 Å, respectively (Fig. 5). These two amino acids were active sites for binding to pantothenate.[9,27] Bond stabilization was also formed through binding the ZVT with Arg238 *via* hydrogen bonding. In addition, the hydrophobic interaction of ZVT with Met242 in the presence of a benzene ring also played a role in stabilizing the bonding of the ZVT––*Mt*Pank complex. In general, all the tested compounds showed the same inhibitory mechanism. All tested compounds were in the binding pocket and bound to

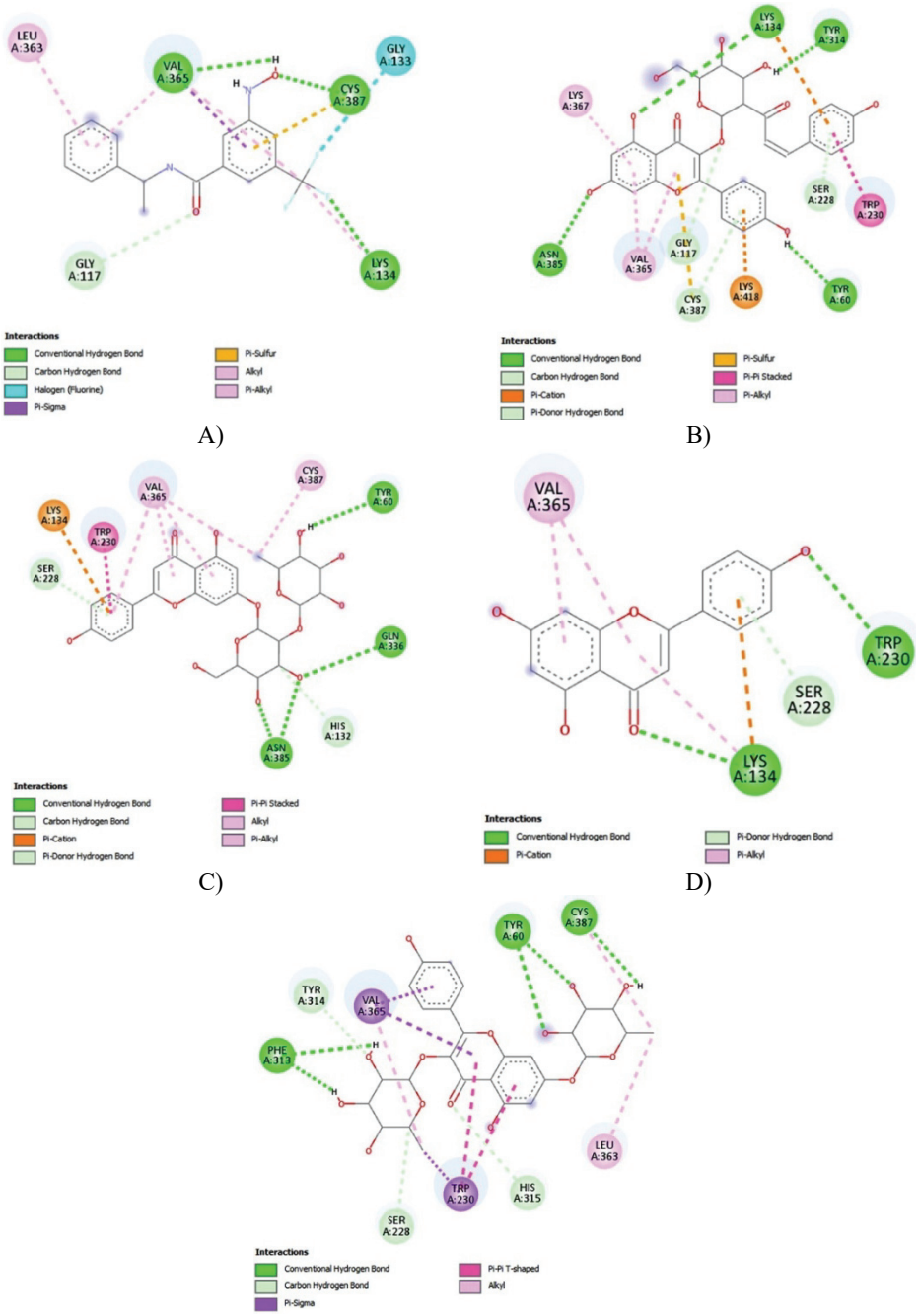


Fig 4. Chemical interaction of *Mt*DprE1 enzyme with: A) 0T4 and compounds: B) F10, C) F7, D) F13 and E) F8.

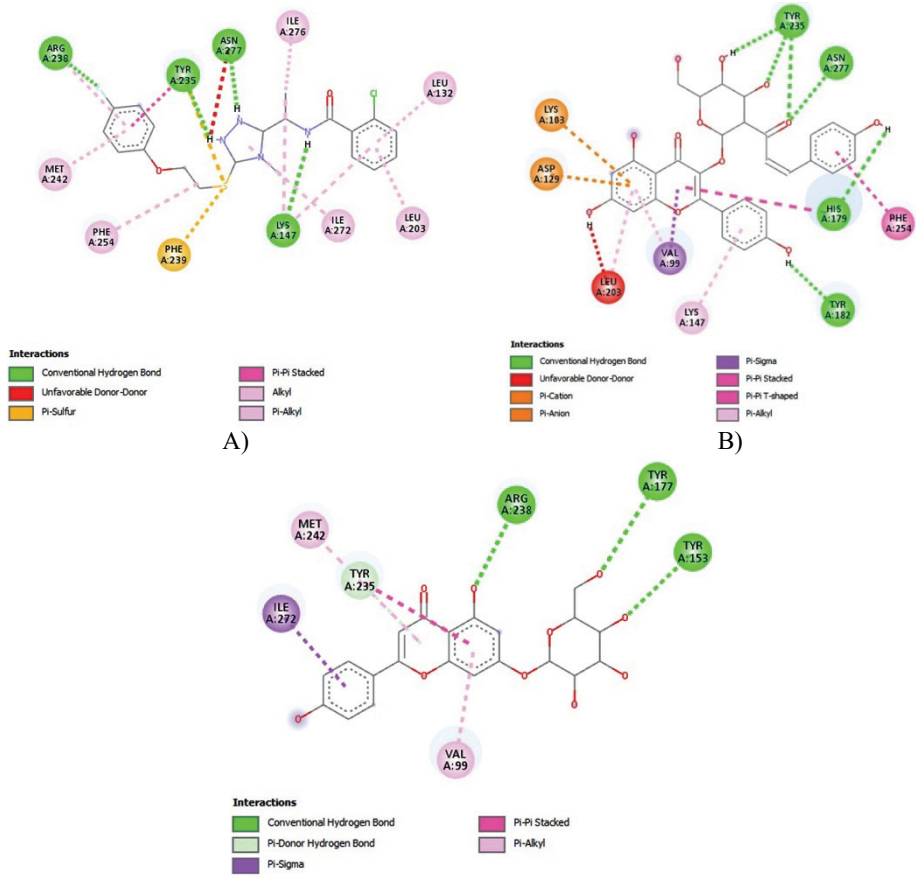


Fig 5. Chemical interaction of *Mt*Pank enzyme with: A) ZVT and compounds: B) F10 and C) F6.

the active site of the *Mt*Pank enzyme. For the compound F10, the hydroxyl group of the glucose substituent facilitated hydrogen bonding with Tyr235. In contrast, the Asn277 amino acid was bonded by the oxygen atom of the coumaroyl carbonyl group. Compound F6 only binds Tyr235 with a bond length greater than 3.87 Å, which matches the binding energy value and is slightly lower than the compound F10.

It was strongly suspected that the presence of apigenin and kaempferol as core structures contributed to the inhibition of the tested compound against the *Mt*Pank enzyme. This was shown by the interactions of compounds F12 and F13. Visualization of the bond between these two compounds exhibited the presence of binding to two key amino acid residues of the *Mt*Pank enzyme. Compounds F12 and F13 showed very effective binding to Tyr235 and Asn277. This means

that these two compounds were in the binding pocket of *Mt*Pank and had the same inhibitory mechanism. These molecular docking results confirm that compounds F12 and F13 were potentially active as *Mt*Pank enzyme inhibitors.

ADMET-Drug-likeness analysis

The pharmacokinetic properties of the tested compounds were further analyzed using pkCSM web tools to reveal their bioavailability. The results are presented in Table II. In terms of absorption, the highest absorption ability across the intestinal membrane was shown by compounds F12 and F13 with values of >80 %. Furthermore, distribution character was studied through the value of volume of distribution (*VD*), blood–brain barrier permeability (*BBB*) and central nervous system permeability (*CNS*). All of the tested compounds showed moderate (–0.15–0.45) to low (<–0.15) log *VD* values. Compounds F8, F9, F10, F11 and F13 with low log *VD* indicated that these compounds tended to be distributed in plasma. Furthermore, the *BBB* value revealed that all compounds were unable to penetrate the blood–brain barrier (<1.00).[22] The same case was also shown by the *CNS* value of all tested compounds. The analysis showed that all compounds had poor absorption into the central nervous system.

TABLE II. ADMET properties of natural flavonoid compounds from *Delonix regia* leaf; metabolism not found for all compounds in cases of 2D6 and 3A4 substrates CYP and 2D6 inhibitor

ID Compd.	Intestinal absorption, %	Distribution			Metabolism for 3A4 inhibitor	Excretion, log (total clearance in mL min^{-1} kg^{-1})	AMES toxicity
		log (*VD* / L kg^{-1})	log *BBB*	log *CNS*			
F1	21.25	0.01	–2.87	–5.82	No	0.32	Yes
F2	30.96	0.04	–2.80	–5.79	No	0.34	Yes
F3	56.15	0.10	–1.82	–4.51	No	0.69	No
F4	38.87	0.36	–2.58	–5.18	No	0.59	Yes
F5	48.16	0.41	–1.59	–3.62	No	0.42	No
F6	41.10	0.11	–1.52	–4.03	No	0.60	No
F7	30.44	0.03	–2.33	–5.33	No	0.50	No
F8	26.98	–0.40	–1.81	–5.22	No	0.09	No
F9	60.01	–0.24	–2.11	–4.83	No	0.64	No
F10	61.35	–0.98	–1.80	–4.15	Yes	0.14	No
F11	34.94	–0.22	–2.32	–5.29	No	0.60	Yes
F12	84.79	0.01	–1.32	–2.44	Yes	0.62	Yes
F13	91.64	–0.18	–1.05	–2.25	Yes	0.67	No

Cytochrome P450 is an enzyme in charge of drug metabolism, and in this study, the isoforms used were 2D6 and 3A4. The analysis showed that only the compound F4 could act as a CYP3A4 substrate and compounds F10, F12 and F13 were inhibitors of CYP3A4. The next analysis was total clearance, a value describing the drug's ability to be metabolized in the liver and excreted through

the kidney.[28] The results revealed that almost all compounds had a fairly acceptable total clearance value ranging from 0.14–0.67, except for the compound F8, which had a low total clearance value (0.09). Furthermore, the toxicity properties of a drug were also a critical aspect of further drug development. The results of AMES toxicity analysis found that compounds F1, F2, F4, F11 and F12 had mutagenic and carcinogenic properties, but other compounds had the opposite properties. Thus, they had the potential to be further developed as drug candidates.

Drug-likeness evaluation of all tested compounds was then carried out and used as a screening stage based on the Lipinski rule.[29] Table III presents the data from the drug-likeness analysis of all tested compounds. Compounds F5, F12 and F13 did not violate the Lipinski rule. The molecular weight of the compound (*MW* > 500) indicated that the compound had poor absorption ability. Log *P* value described the lipophilic character and all tested compounds had a Log *P* value below the cut-off value (>5).

TABLE III. Drug-likeness of natural flavonoid compounds from *Delonix regia* leaf.

Compound	*MW*	Log *P*	Number of HBA	Number of HBD	*PSA* Å^2	Violation of Lipinski rule	Drug-likeness score
F1	564.49	–0.18	14	10	198.92	3	0.26
F2	578.16	–0.00	14	10	196.75	3	0.36
F3	448.10	0.47	11	7	151.29	2	0.60
F4	610.19	–0.81	15	8	186.70	3	0.94
F5	360.08	1.54	8	5	114.28	–	0.37
F6	432.11	0.91	10	6	135.81	1	0.59
F7	578.16	0.30	14	8	182.25	3	0.81
F8	578.16	–0.71	14	8	179.86	3	0.73
F9	448.10	0.32	11	7	150.41	2	0.82
F10	578.14	1.71	12	7	167.32	3	0.63
F11	592.18	0.82	14	7	172.17	3	0.70
F12	286.05	1.61	6	4	87.13	–	0.50
F13	270.05	3.22	5	3	73.57	–	0.39

Referring to the Lipinski rule, hydrogen bonding acceptor (*HBA*) must be below 10, and hydrogen bonding donor (*HBD*) must be below 5.[30] These two values were correlated with the polar surface area (*PSA*) value. When a compound has a violation of *HBA* and *HBD*, it tends to have low bioavailability with a *PSA* value greater than 140.00 Å^2. All the tested compounds had *HBA* > 10 and *HBD* > 5 except compounds F5, F12 and F13. For the drug-likeness score, all compounds gave a fairly acceptable value with a range of 0.26–0.94. Compound F4 had the largest drug-likeness score, namely 0.94. Unfortunately, this compound also had toxic properties based on the results of the AMES toxicity analysis. Compared with the results of ADMET analysis and drug-likeness analysis, the compound F12 had toxic properties while the compound F5 had poor ads-

orption characteristics. As a result, compound F13 as the lead compound is further investigated for its stability against all 3 enzymes.

Molecular dynamic simulation

Molecular dynamics using the CABS Flex 2.0 server was performed and provided a root mean square fluctuation (*RMSF*) graph to evaluate the stability of compound F13 key important enzymes of *M. tuberculosis* (Fig. 6). As reported by Jamroz *et al*., *RMSF* analysis using the CABS Flex 2.0 server demonstrated significant correlation with NMR spectroscopy analysis.[31] As shown in Fig. 6, compound F13 formed the most stable complex against the *Mt*KasA enzyme. It was found that the fluctuating value of all amino acid residues was 1–3 Å.[32] The F13–*Mt*DprE1 complex showed high fluctuations at residue numbers 17, 60, 166, 258 and 284. Meanwhile, high fluctuation levels for residue numbers 85, 248, 249, 250, 251 and 252 were found in the F13–*Mt*Pank complex. The high

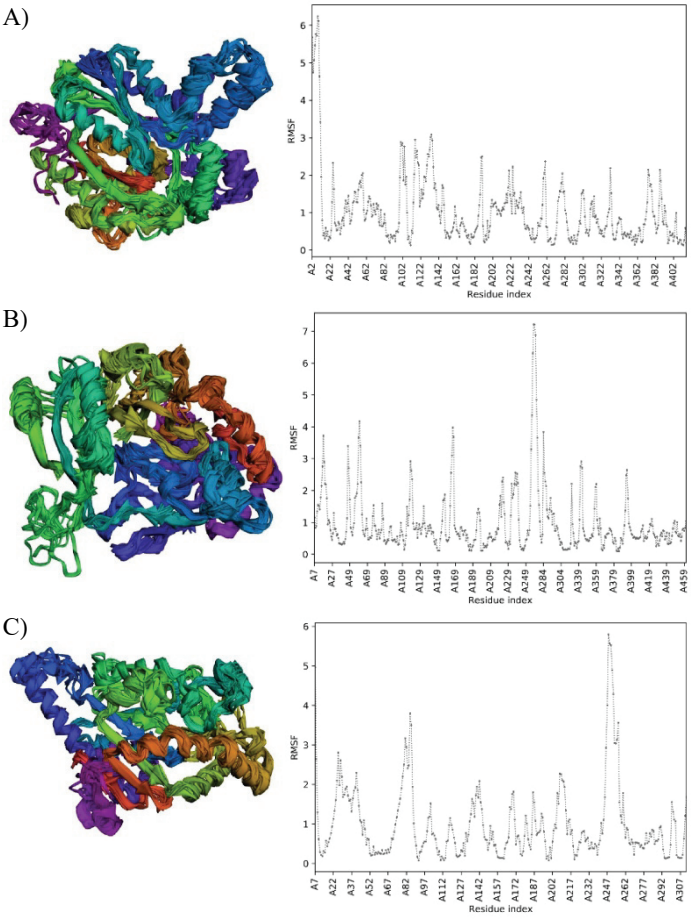


Fig 6. Multimodel and RMSF of compound F13 against: A) *Mt*KasA, B) *Mt*DprE1 and C) *Mt*Pank enzymes.

fluctuating value means that the flexibility of amino acid residues is very considerable. This reduces the stability of the complex.

The results of *in silico* studies indicated that compound F13 was the most effective compound in inhibiting *M. tuberculosis* by forming a stable complex with the *Mt*KasA enzyme. However, there is still a need to confirm compound F13 activity *in vitro* and *in vivo* through laboratory experiments.

CONCLUSION

In silico study of natural flavonoid compounds from *Delonix regia* leaves extract showed that all tested compounds had antimycobacterial properties, based on *in silico* analysis. ADMET and drug-likeness screening showed that among all the existing test compounds, only compound F13 had the potential to be studied further as an antimycobacterial candidate with binding energy values for *Mt*KasA, *Mt*DprE1 and *Mt*Pank of–35.98, –34.31 and –33.05 kJ/mol, respectively. The molecular dynamics simulation results suggested that compound F13 formed the most stable complex against the *Mt*KasA enzyme compared to *Mt*DprE1 and *Mt*Pank.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12064, or from the corresponding author on request.

ИЗВОД

ПРИРОДНИ ФЛАВОНОИДИ У ЛИСТОВИМА *Delonix regia* КАО АНТИМИКОБАКТЕРИЈСКИ АГЕНСИ: *IN SILICO* СТУДИЈА

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Отпорност на многоструке лекове (MDR) и екстензивна отпорност на лекове (XDR) као резултат сталне употребе антибиотика охрабрује развијање нових антимикобактеријских лекова. У овом раду је проучавано 13 флавоноидних једињења из листова биљке *Delonix regia* због њихових инхибиторних особина за *Mt*KasA, *Mt*DprE и *Mt*Pank, који су значајни ензими код *Mycobacterium tuberculosis*, као и њиховог молекулског докинга, молекулске динамике, те предвиђања ADMET сличности са лековима. Резултати студије молекулског докинга откривају да је једињење F13 (apigenin) било најпотентније једињење пошто је било у стању да веже већину аминокиселина на које указује нативни лиганд сваког ензима. Студије молекулске динамике су показале да једињење F13 формира стабилан комплекс са *Mt*KasA. Из резултата ADMET-анализе сличности са лековима закључује се да је једињење F13 оно које највише обећава. Све у свему, једињење F13 има потенцијал да се користи као терапија лечења *M. tuberculosis*.

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