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Research Article

In Silico Active Compounds of *Musa troglodytarum* L. as Antibiotic Candidates for Tuberculosis

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ABSTRACT
The primary approach for managing tuberculosis involves the use of antibiotics, such as isoniazid. Over time, genetic mutations give rise to bacterial resistance against synthetic medications. As a result of this phenomenon, the aforementioned impacts can be mitigated through the utilization of bioactive compounds derived from naturally occurring substances, such as mature bananas (Musa troglodytarum L*). The objective of this work was to assess the pharmacokinetics and physicochemical properties of Ranggap bananas, as well as their binding affinity on the 4KL9 receptor, in order to anticipate potential toxicity using the in silico molecular docking approach. The findings indicate that the Ranggap banana contains Fumaric acid and Benzoic Acid compounds that exhibit a stronger binding affinity for the 4KL9 receptor compared to isoniazid. The ligand's binding affinity is more negative by -4.8 kcal/mol and -5.4 kcal/mol, satisfying Lipinski's five laws, including a molecular weight of 116.072 g/mol and 122.123 g/mol, Log p values of 0.2882 and 1.3848, HBA values of 2 and 1, and HBD values of 2 and 1. Additionally, the compounds demonstrate a favourable ADME profile (Absorption, Distribution, Metabolism, and Excretion) and fall within toxicity classes 3 and 4, which are considered safer than isoniazid. Consequently, these two compounds possess the potential as tuberculosis drugs that minimise adverse effects.*

Keywords: *Compounds; Infectious Disease; Molecular Docking; Mycobacterium tuberculosis.*

Introduction

Tuberculosis is among the ten primary causes of mortality and holds the first position as the cause of mortality attributed to a single infectious agent, surpassing HIV/AIDS. According to the [1], a significant number of individuals are afflicted with tuberculosis on an annual basis. According to Sia and Rengarajan [2], the global incidence of tuberculosis (TB) in 2016 was around 10.4 million cases, with a mortality rate of nearly 1.7 million individuals. According to the [1], the mortality rate among those afflicted with tuberculosis (TB) decreased from 23% in 2000 to 16% in 2017. The condition known as tuberculosis (TB) is characterised by the gradual multiplication of bacteria and the development of necrotic tissue in the lungs. It is important to note that the presence of cavity lesions, which facilitate the passage of germs, is not always a feature of TB [3]. The World Health Organisation (WHO) defines latent tuberculosis infection (LTBI) as a condition characterised by a sustained immune response to antigens of *Mycobacterium tuberculosis*, in the absence of clinically apparent active tuberculosis,

and with limited or no detectable bacillary replication due to immunological control [3].

In the year 2022, the Ministry of Health, in collaboration with healthcare professionals, successfully identified over 700,000 instances of tuberculosis (TBC). This numerical value represents the highest recorded figure since the inception of TBC as a national priority. In Indonesia, the prevalence of Tuberculosis (TB) is the third highest globally, following India and China. Annually, there are approximately 824 thousand reported cases of TB, resulting in 93 thousand fatalities. This translates to an average of 11 deaths occurring every hour. According to the Global TB Report for 2022, the age group with the highest incidence of tuberculosis (TB) cases is the productive age cohort, specifically individuals between the ages of 25 and 34. According to the [4], the majority of tuberculosis (TB) cases in Indonesia are concentrated within the productive age bracket, with a particular emphasis on individuals between the ages of 45 and 54.

Isoniazid, a synthetic medication, is frequently employed as an antibiotic for the treatment of tuberculosis (TB). Isoniazid (INH) is considered a crucial first anti-tuberculosis drug due to its potent early bactericidal efficacy. Currently, the prevalence of resistance to isoniazid (INH) as a standalone treatment or in combination with other medications has become the predominant form of resistance observed in the context of anti-tuberculosis (TB) treatments. The prevalence of isoniazid (INH) resistance in the absence of concomitant rifampicin (RIF) resistance is reported to be 7.1% among individuals with newly diagnosed tuberculosis (TB) cases, and 7.9% among individuals with previously treated TB cases, based on global data. Regrettably, there has been no decrease observed in the worldwide prevalence of resistant isoniazid-resistant tuberculosis (INH-TB) [5].

Drug resistance in Mycobacterium TB arises as a result of spontaneous genetic changes. The development of acquired drug resistance typically arises in scenarios where there is a substantial presence of bacterial populations, such as within the lung cavity, or when insufficient treatment combinations or dosages are administered. Numerous studies have documented risk factors associated with the development of isoniazid (INH) resistance, with a predominant finding being a robust association between prior tuberculosis (TB) treatment and INH resistance. According to [5], a study examining the prevalence of drug-resistant tuberculosis (TB) in the United States identified several risk variables associated with isoniazid (INH) resistance. These risk factors include a personal history of TB, foreign birth, and belonging to the Pacific Islander or Asian ethnic groups.

In light of the emergence of bacterial resistance to isoniazid antibiotics, there is a pressing need for scientific investigations aimed at identifying alternative natural compounds that can effectively suppress the growth of *Mycobacterium tubercolosis* bacteria, hence replacing the therapeutic efficacy of isoniazid. The use of natural substances is imperative due to the exorbitant cost associated with synthetic pharmaceuticals and the potential adverse effects that can pose risks to one's health [6]. The ripe banana is being considered as a potential candidate for a novel tuberculosis (TB) medication due to its natural composition. Ranggap bananas are a variety of bananas that are mostly located in the vicinity of Mount Galunggung and are currently facing the threat of near extinction. According to Hernawati *et al* [7], Ranggap bananas exhibit a unique characteristic in that their bunches are oriented towards the sky, in contrast to the typical downward-facing orientation of banana bunches. This distinctive feature has led to the designation of these bananas as "banana Ranggap". Interestingly, when inverted, the bunches of

Ranggap bananas bear a resemblance to woven bamboo structures traditionally used for confining hens. According to Ploetz in [7], the sap of this particular banana exhibits a range of colours from pink to dark purple. The chemicals utilized in this investigation were 3-methylbutanal, 2-hexenal Fumaric Acid, and Benzoic Acid, all derived from Ranggap bananas.

In order to assess the potential of chemicals found in Ranggap banana to suppress the activity of *Mycobacterium tubercolosis*, it is important to conduct initial in silico testing utilizing molecular docking techniques. Molecular docking is a computational technique utilized in drug design that employs the principles of molecular interactions to simulate and predict the binding mechanism and affinity between receptors and ligands [8]. The utilization of in silico approaches is favoured due to its cost-effectiveness and expeditiousness in generating research findings [9]. The receptor employed in this investigation was identified as 4KL9. The Protein Data Base (PDB) entry 4KL9, which pertains to the pathogen Mycobacterium TB, includes natural ligands, specifically NDP and P33. Previous research has examined the molecular docking of Mycobacterium TB, specifically focusing on the protein dyhidrofolate reductase, using natural chemicals. The target protein used in this investigation was 4KL9, with the P33 ligand [10]. The 4KL9 protein is a protein that can be targeted to inhibit the activity of *Mycobacterium tuberculosis*. It has a low affinity for the original ligand. The presence of similar residues in the 4KL9 protein between the original ligand and natural antibacterial compounds suggests that *Mycobacterium tuberculosis* has high antibacterial activity [11]. The primary focus of this study is to examine the docking interactions between the protein 4KL9 and natural compounds, specifically the Ranggap bananas.

Therefore, the objective of this study was to investigate the physicochemical and pharmacokinetic properties of Ranggap banana compounds in their potential to inhibit the activity of *Mycobacterium tuberculosis*. Additionally, the study aimed to assess the toxicity level of the active compound of Ranggap banana through in silico analysis. Lastly, the study aimed to determine the affinity of the active banana compound for 4KL9 receptors using in silico methods.

Materials and methods

The primary objective of the proposed research is to conduct an in-silico analysis utilizing the molecular docking technique. This analysis will be performed on the active chemicals found in banana-like fruits, specifically *Benzoic Acid and Fumaric Acid***,** in order to investigate their interactions with Mycobacterium TB. The factors under consideration encompassed the prognostications of physicochemical characteristics, pharmacokinetic attributes, toxicity profiles, and energy binding affinities about the active constituents found in Ranggap bananas. Based on the obtained data, it may be inferred which chemicals possess a greater potential to be considered as medication candidates for tuberculosis (TB).

The Process of Searching for and Downloading Ligands and Receptors

The identification of the chemicals present in Ranggap bananas was achieved through autonomous investigations conducted within a controlled laboratory setting. The aforementioned chemical was identified in the outcomes of the Ranggap banana LMS by pkCSM Online Tool with parameter of Lipinski five law parameters experiment. The ligands of the test compounds were obtained from PubChem as a sdf file. Subsequently, the SMILES code of each compound was documented for the purpose of physicochemical, pharmacokinetic, and toxicity prognostications. The identification of the receptor was accomplished through a comprehensive review of relevant

literature, specifically focusing on prior research publications that used the 4KL9 receptor as a means to facilitate the exploration for potential tuberculosis medication candidates. The process of downloading the 4KL9 protein is carried out within the RCSB PDB platform, where it is made available as a file with the .pdb extension.

The Synthesis of Ligands for the Test Compound

The ligand preparation process involves acquiring the 3D structure of each ligand test compound from the PubChem website in the .sdf file format. Subsequently, the ligands were saved in.pdb file format utilizing the Biovia Discovery Studio Visualizer 2021 software. The ligands employed in this investigation were Fumaric acid and Benzoic acid, both of which are present in Ranggap Bananas. Additionally, the antibiotic comparator utilized as isoniazid. The ligand format is transformed into the .pdb file format via the Biovia Discovery Studio Visualizer 2021 software, enabling compatibility with the Autodock Tool 1.5.7 software for subsequent analysis.

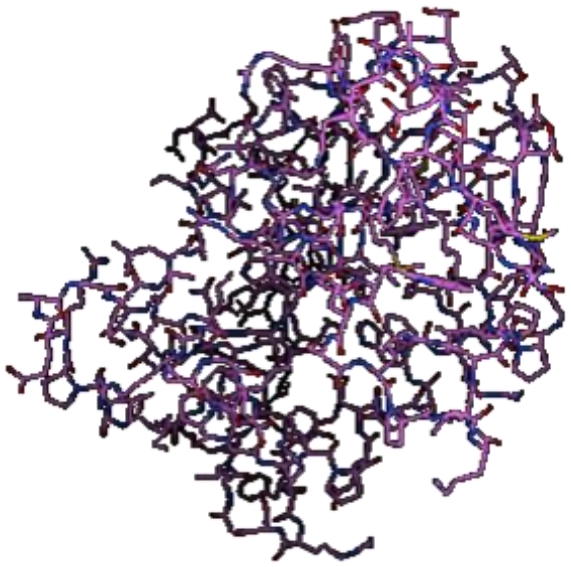


Figure 1. 4KL9 Receptor Preparation Results Source: Receptor Preparation Results Personal Document via AutoDock Tool 1.5.7

Preparation of Receptors

The target receptor is dissociated from the water molecule, and the native ligand bound to it is detached with the assistance of Biovia Discovery Studio Visualizer 2021. Hydrogen is subsequently introduced in a polar-only configuration, and the merging of non-polar entities is performed utilizing AutoDock Tool 1.5.7. Subsequently, the data is stored in the format of pdbqt file. The outcomes of the receptor preparation are illustrated in Figure 1 presented thereafter.

Data Analysis

Measurement of the affinity of the active compound of Ranggap banana for the target receptor PDB ID 4KL9 was measured based on the comparison of binding relationship, the RMSD (Root Mean Square Deviation) value consisting of RMS l.b (lower bound) and RMSD u.b (upper bound) as well as the interaction between the ligand and its target receptor. The physicochemical predictions are measured based on Lipinski's rule of five. Namely, a compound is said to be a drug candidate if it has a molecular weight (BM) <500, logarithm of the octanol partition coefficient (LogP) <5, hydrogen bond donor (HBD) <5, hydrogen bond acceptor (HBA)<10, and no more than two errors/violations with the help of the pkCSM online tool. Predictive analysis of pharmacokinetic properties was measured based on ADME (Absorption, Distribution, Metabolism, and Excretion) indicators with the help of the pkCSM online tool. As for the prediction of toxicity, using the benefit of the ProTox online tool to measure the LD50 value and toxicity class and using the pkCSM online tool to measure mes toxicity and hepatotoxicity. This study utilizes the Ames test to assess the genotoxicity potential of the antibacterial compound. The test involves measuring and identifying mutations in bacterial strains that disable genes responsible for synthesizing crucial amino acids. Consequently, bacteria are

able to thrive in specific culture media containing amino acids. This test reveals the toxicity by the partial or total lack of a bacterial background lawn or a large dose-related drop in revertant colony numbers, compared with the lower dose levels and concurrent vehicle control accounting for the laboratory historical control range. The resulting data will be analyzed descriptively.

Results and Discussion

Results

1. Physicochemical Prediction

The results of the prediction analysis of the physicochemical properties of the test compound ligand are presented in (Table 1), and the pharmacokinetics (Table 2), toxicity prediction of the test compound and Isoniazid comparison compound (Table 3).

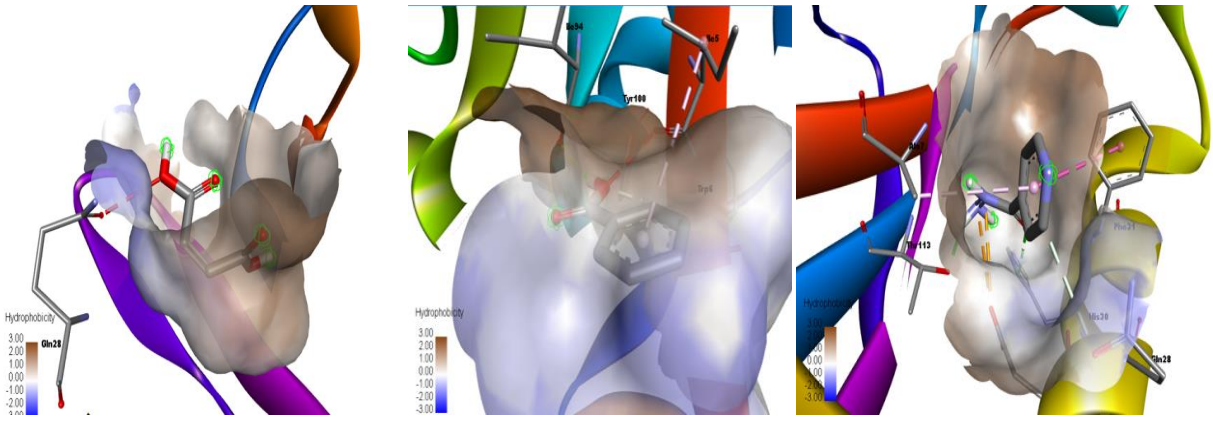
Table 1. Prediction Results of Physicochemical Properties of Compounds using the pkCSM Online Tool

Compounds	Lipinski's Five Law Parameters*				Application of Lipinski's Five Laws
	BM (g/mol)	Log P	HBA	HBD	
Fumaric Acid	116.072	-0.2882	2	2	Yes, 0 Error
Benzoic Acid	122.123	1.3848	1	1	Yes, 0 Error
Isoniazid (comparison antibiotic)	137.147	-0.3149	3	2	Yes, 0 Error

Notes *: Explanation of Lipinski's Legal Maximum Limit: BM (*Molecular Weight* <500), Log P (*Partition Coefficient* <5), HBA (*Hydrogen Bond Acceptor* <10), HBD (*Hydrogen Bond Donor* <5)

2. Molecular Docking

3D visualization of the interaction between the test compound and isoniazid with the 4KL9 receptor can be seen in Figure 2. The results of molecular docking in the form of binding affinity and RMSD L.B and RMSD U.B values can be seen in Table 4.



a b c

Figure 2. 3D visualization of the test compound's ligand with 4KL9 receptors, (a) 4KL9-Fumaric Acid; (b) 4KL9-Benzoic Acid; and (c) 4KL9-Isoniazid Source: Personal Documentation from Discovery Studio Visualizer 2021

3. Pharmacokinetic Predictions

The results of the pharmacokinetic properties prediction analysis are detailed in Table 2.

Table 2. Predictions of the Pharmacokinetic Properties of the Test Compounds

Compounds	Absorptions				Distributions			Metabolisms				Excretion	
	Intestinal absorption (%)	Solubility in Water (Log mol/L)	Permeability CaCO2 (Log Papp in 10^{-6} cm/s)	VDss (logL/kg)	Permeability BBB (log BB)	Fraction unbound (FU)	Permeability CNS (Log PS)	Substrate CYP2D6	Substrate CYP3A4	Inhibitor CYP2D6	Inhibitor CYP3A4	Renal OCT2	Total Clearance (log ml/min/kg)
Fumaric Acid	71.771	-0.642	0.563	-1.026	-0.127	0.644	-3.046	No	No	No	No	No	0.89
Benzoic Acid	100	-1.738	1.707	-1.64	-0.22	0.523	-2.002	No	No	No	No	No	0.707
Isoniazid (antibiotic compared)	92.601	-1.6	0.52	-0.352	0.002	0.728	-3.351	No	No	No	No	No	0.722

4. Toxicity Prediction

The results of the toxicity prediction of the test compound and the Isoniazid comparison compound can be seen in Table 3.

Table 3. Toxicity Prediction Results

No	Compounds	Toxicity			
		LD_{50} (m/kg)*	*Ames Toxicity***	*Hepatotoxicity***	Toxicity Class *
1	Fumaric Acid	1626	No	No	4
2	Benzoic Acid	290	No	No	3
3	Isoniazid (antibiotic compared)	133	No	No	3

Notes: (*) using *Protox Online Tool*, (**) using *pkCSM Online Tool*

Table 4. Molecular Docking Results of Test Compounds and Comparative Drugs at 4KL9 Receptors

No.	Ligand	Conformation	*Binding Affinity* (kcal/mol)	*dist. from the best mode*	
				rmsd l.b.	rmsd u.b.
1	Fumaric Acid	1	-4.8	0.000	0.000
		2	-4.8	-1.853	2.295
		3	-4.7	12.877	13.542
		4	-4.5	10.963	11.938
		5	-4.5	12.115	12.974
		6	-4.4	1.850	3.156
		7	-4.4	1.909	2.666
		8	-4.3	2.145	4.480
		9	-4.3	1.596	3.131
2	Benzoic Acid	1	-5.4	0.000	0.000
		2	-5.3	4.402	6.275
		3	-5.3	16.205	17.866
		4	-5.1	4.575	5.843
		5	-5.0	2.926	3.744
		6	-5.0	12.081	13.093
		7	-4.9	5.614	7.358
		8	-4.8	11.553	12.840
		9	-4.6	3.412	4.140
3	Isoniazid (antibiotic compared)	1	-5.6	0.000	0.000
		2	-5.4	13.386	14.255
		3	-5.4	2.087	2.702
		4	-5.3	2.266	2.605
		5	-5.2	7.297	8.552
		6	-5.0	2.633	4.520
		7	-5.0	6.049	7.271
		8	-5.0	6.828	8.417
		9	-5.0	3.082	3.664

5. Ligand and Receptor Interactions

Ligand interactions with amino acids can occur in the area of protein binding sites. A 2D visualization of the interaction between the test compound and isoniazid with the 4KL9 receptor can be seen in Figure 3. The results of the amino acid interaction of the test compounds with the 4KL9 receptor are shown in Table 5.

Table 5. Results of Amino Acid Interactions of the Test Compounds at the 4KL9 Receptor

No	Compounds	Amino Acid Interactions
1	Fumaric Acid	ILE A5, TRP A6, ALA A7*, ASP A27*, HIS A30*, PHE A31*, THR A113*, dan GLN A28*.
2	Benzoic Acid	ALA A7*, ILE A20, ASP A27*, GLN A28*, PHE A31*, GLY A95, ILE A94, TYR A100, TRP A6, dan ILE A5
3	Isoniazid (antibiotic Compared)	ASP A27, HIS A30, THR A113, GLN A28, PHE A31, dan ALA A7

(*) : the same amino acid residue as the Isoniazid control

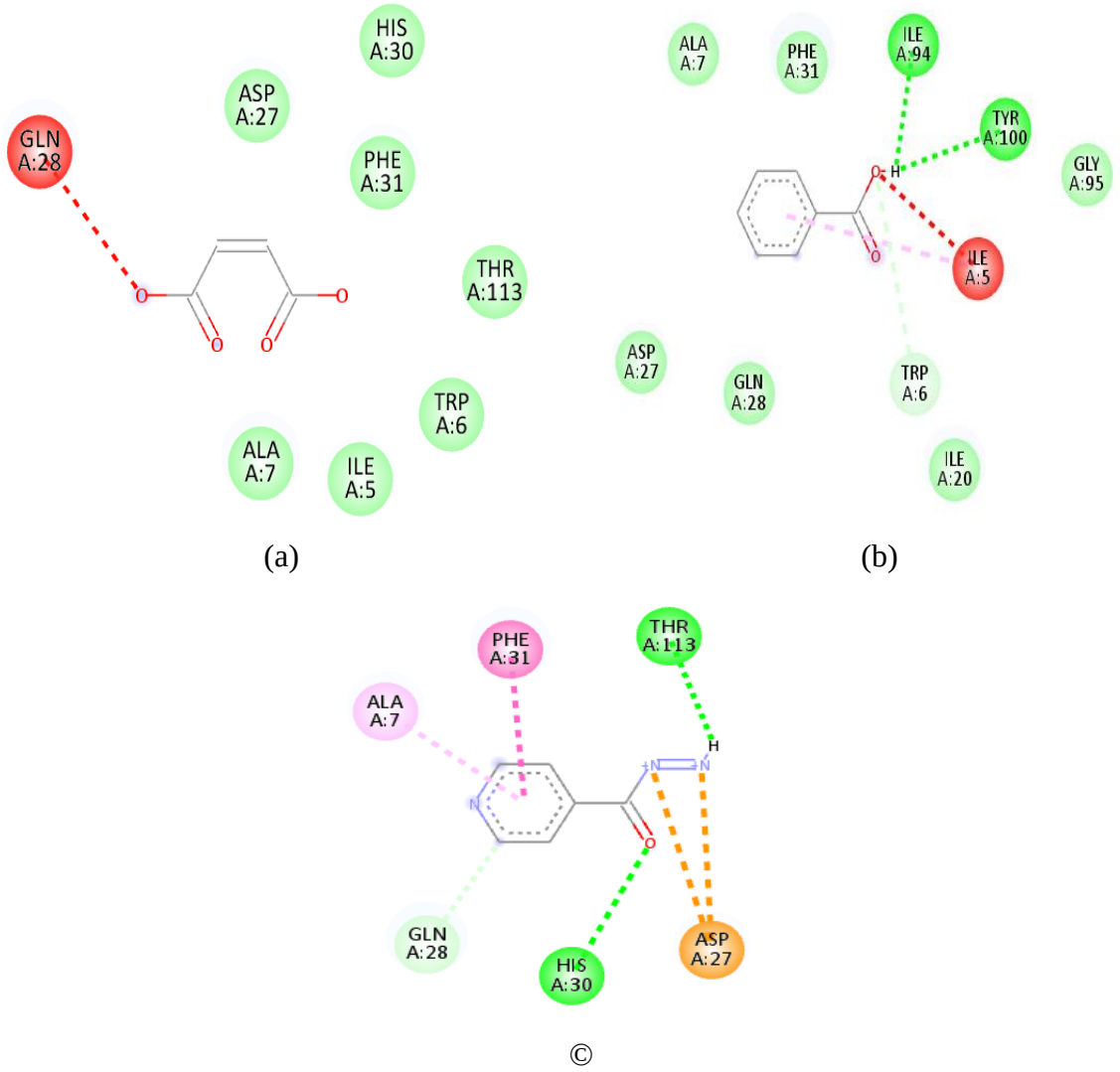


Figure 3. 2D visualization of the test compound's ligand with 4KL9 receptors: (a) 4KL9-Fumaric Acid; (b) 4KL9-Benzoic Acid; and (c) 4KL9-Isoniazid (software used to this visualization)

Discussions

According to Gu *et al* [11], the physicochemical prediction findings indicate that Fumaric acid and Benzoic Acid adhere to Lipinski's five principles, which include a molecular weight below 500, a Log P value below 5, a number of hydrogen acceptor bonds (HBA) below 10, and several hydrogen donor bonds (HBD) below 5. This implies that the substance is anticipated to exhibit high absorption rates, favourable permeability characteristics, and substantial oral bioavailability. Based on the principles outlined in Lipinski's five laws and the findings derived from the investigation, it may be inferred that the two compounds possess the capacity to be developed into pharmaceutical agents. Both compounds possess molecular weights that are lower than that of Isoniazid. This implies that both chemicals exhibit a high degree of permeability across biological membranes. There exists a positive correlation between molecular weight and the level of difficulty in traversing biological membranes. According to Ruswanto [12], substances possessing a molecular weight over 500 have a substantial molecular size, hence indicating potential challenges in traversing biological

membranes. Upon ingestion of a drug, drug molecules come into contact with various biomembranes, including circulating macrophage cells and blood vessel endothelium. This interaction often occurs by passing through the phospholipid bilayer and pores of the membrane, or through protein-containing transporters. According to the principle of molecular similarity, it is generally expected that oral drugs that are successful and available in the market should have a structural similarity to at least one intermediate metabolite. This is because the natural substrates of these transporters are usually intermediate metabolites [13]. The physiological impacts are uncertain at present.

The log P value in physicochemical predictions (Table 1), is associated with the lipophilicity or hydrophobicity of chemical compounds, which refers to their capacity to readily dissolve in fats, oils, lipids, and non-polar solvents [12]. Both compounds exhibit a greater Log P value compared to Isoniazid, indicating their enhanced ability to permeate biological membranes and exhibit stronger receptor binding affinity in comparison to Isoniazid. The biological activity of a pharmacological molecule is influenced by the presence of hydrogen bond acceptor and hydrogen bond donor functionalities. The chemical-physical properties of compounds, such as boiling point, melting point, solubility in water, ability to form chelates, and acidity, can undergo modifications that impact the biological activity of compounds due to hydrogen bonding [12]. Both test compounds containing Isoniazid had values for hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) that were found to be below the established maximum limit.

The pharmacokinetic prediction (Table 5), was conducted through the assessment of the ADME profile, encompassing the processes of absorption, distribution, metabolism, and excretion. To examine the absorption profile of Calcium Carbonate ($CaCO_2$), it is essential to consider the factors associated with intestinal absorption, water solubility, and permeability. According to Chander *et al* [13], a compound's absorption can be considered favourable if its intestinal absorption value exceeds 80%, while it is deemed inadequate if it falls below 30%. Both the chemicals found in Ranggap bananas and the comparator antibiotics exhibit favourable absorption values. The efficacy of oral medication can be seen from administration in terms of intestinal absorption and subsequent entry into the bloodstream [14]. According to a study conducted by [15], drugs that exhibit solubility in water tend to have superior absorption rates compared to those that are soluble in fat. According to [16], compounds exhibit low solubility when their water solubility value is less than -6. Both test compounds exhibit significant water solubility.

The use of $CaCO_2$ single-layer cells derived from human colon adenocarcinoma is prevalent in in vitro models of the human intestinal mucosa to predict the absorption of orally delivered medicines. A compound is considered to possess a significant level of $CaCO_2$ permeability if its value exceeds 8×10^{-6} cm/s. However, when utilizing pkCSM, this value will be converted into a predicted value greater than 0.90 [15]. Benzoic acid compounds exhibit a significantly higher permeability value for $CaCO_2$ in comparison to isoniazid. This suggests that these compounds possess the capacity to effectively traverse the intestinal cell membrane.

The distribution profile encompasses an examination of various parameters, including Volume Distribution Steady-state (VDSs), permeability of the Brain Blood Barrier (BBB), fraction unbound, and permeability within the Central Nervous System (CNS). When considering the volume of distribution (VDss), it can be observed that 3-methylbutanal and 2-hexenal exhibit log VDss values below -0.15, namely -0.047 and 0.018 respectively. These chemical

exhibits uniform distribution, hence enabling the attainment of equivalent concentrations in blood plasma. According to Pires *et al* [15], a compound's VDss value can be categorized as low if the logarithmic VDss value is less than -0.15, while it is regarded high if the logarithmic VDss value exceeds 0.45. According to Pires *et al* [15], a compound's ability to effectively penetrate the blood-brain barrier is enhanced when its log BB value exceeds 0.3. Conversely, compounds with a log BB value lower than -1 demonstrate inadequate distribution across the blood-brain barrier. It is anticipated that Fumaric Acid, Benzoic Acid, and Isoniazid have comparable distribution characteristics, resulting in equilibrated concentrations within the blood plasma. According to Pires *et al* [15], an inverse relationship exists between the unbound fraction parameter and the ability of a protein to traverse the cell membrane or undergo diffusion. Specifically, as the protein becomes more bound in the bloodstream, its efficiency in crossing the cell membrane or diffusing decreases. The unbound fraction value of the two active compounds found in Ranggap banana is lower compared to that of the comparator antibiotics. This suggests that these compounds are likely to exhibit efficient cell membrane crossing or diffusion capabilities. Central nervous system (CNS) permeability refers to the capacity to effectively traverse the barriers of the central nervous system. According to a study conducted by [15], a logPS value more than -2 indicates the ability to permeate the central nervous system (CNS), whereas a logPS value lower than -3 indicates the inability to reach the central nervous system. Both chemicals are hypothesized to exhibit little ability to cross the blood-brain barrier.

The metabolic profile is characterized by the presence or absence of cytochrome P450 inhibition, particularly targeting the CYP2D6 and CYP3A4 isoforms. According to [16], chemicals that serve as substrates for CYP450 enzymes undergo metabolism by these enzymes, whereas substances that act as inhibitors can attenuate their metabolic activity. According to Mutiara [17], the presence of drugs that inhibit cytochrome P450 enzymes (CYP inhibitors) can lead to an elevation in their toxicity levels. The two test substances did not exhibit the characteristics of being substrates or CYP inhibitors, thus indicating that their toxicity levels were not expected to escalate (Table 2).

The excretion profile can be determined by examining the parameters of Total Clearance (CLOT) and Renal Organic Cation Transporter 2 (OCT2). The CLTOT parameter is employed to estimate the rate at which a molecule is eliminated from the body, as indicated by a study conducted by [15]. Fumaric acid exhibits a greater CLTOT value in comparison to isoniazid, indicating a more rapid excretion of the substance from the human body. According to Pires *et al* [15], it is anticipated that the compounds under investigation do not exert any influence on OCT2 substrates. Consequently, it can be inferred that these compounds are unlikely to do any harm to OCT2 substrates when administered concurrently with OCT2 inhibitors. The findings derived from the analysis of the OCT2 parameter indicate that none of the chemicals examined exhibit any significant side interactions.

By the classification of toxicity classes outlined by the Globally Harmonised System (GHS), the LD50 number can serve as an indicator for predicting toxicity. Specifically, a higher LD50 value suggests a larger degree of tolerability or presence of the substance within the body. Fumaric Acid exhibits a greater LD_{50} value in comparison to isoniazid, except Benzoic Acid. It is noteworthy that Benzoic Acid belongs to the same class as Isoniazid, specifically class 3 (Table 3). According to the Ames toxicity parameter, both substances exhibit unfavourable characteristics. This chemical is characterized by its mutagenic properties,

hence precluding its classification as a carcinogen. In the study conducted by [15], it was shown that none of the compounds exhibited hepatotoxicity, indicating a lack of liver toxicity. Based on the comprehensive findings, it is evident that Fumaric acid, classified as a compound of class 4, exhibits a comparatively higher level of safety, lacks mutagenic properties, and does not induce toxicity in the liver. The toxicity of this molecule is superior to that of the comparative antibiotic, specifically isoniazid.

The acceptable threshold for the root means square deviation (RMSD) value in structural conformation alignment is less than 3. According to [18], the alignment value is considered better when it approaches zero, with an optimum value being less than 2. All of the compounds that were subjected to testing were deemed genuine due to their conformation 1 exhibiting a value of 0. Nevertheless, it was noted that not all ligands exhibiting an interaction posture between the ligands and receptors were deemed legitimate. Ligands exhibiting favourable interactions in structurally plausible conformations were identified within the ligand set associated with the Fumaric Acid test chemical. The ligands present in the compounds under investigation exhibited binding affinities superior to that of isoniazid. Specifically, Fumaric Acid and Benzoic Acid demonstrated sequential binding affinity values of -4.8 kcal/mol and -5.4 kcal/mol, respectively.

In the interim, it is observed that the binding affinity of isoniazid is -5.6 kcal/mol. Evidence demonstrates that fumaric and benzoic acid exhibit lower binding affinity for the 4KL9 receptor compared to Isoniazid (Figure 2 and Figure 3). According to Yahmin *et al* [19], ligands with more negative binding affinity values are considered to be the most optimal. According to Prasetiawati *et al* [20], amino acid residues that are identical to those found in the reference molecule or natural ligand can exhibit comparable biological activity. Fumaric acid exhibited the highest number of residue similarities with isoniazid, specifically ALA A7, ASP A27, HIS A30, PHE A31, THR A113, and GLN A28, based on the comparison of amino acid residues. Homologous amino acid residues share a common mechanism for transporting pharmacological molecules across biological membranes, potentially involving transporter proteins.

Conclusions

The Ranggap bananas (*Musa troglodytarum* L.) contains pharmacokinetic chemicals, namely Fumaric Acid (FA) and Benzoic Acid (BA), which exhibit potential as therapeutic agents for tuberculosis patients. Both of these compounds have the advantage of mitigating the potential for microbial resistance to a greater extent when compared to the antibiotic Isoniazid. The outcomes of physicochemical compound prediction tests indicated that both compounds had a molecular weight below 500 g/mol, a Log P value below 5, less than 10 hydrogen bond acceptors (HBA), and less than 5 hydrogen bond donors (HBD). Subsequent analysis of the ADME profile (Absorption, Distribution, Metabolism, and Excretion) revealed that both compounds exhibit notable water solubility and demonstrate facile absorption across intestinal cell membranes and blood vessels. Furthermore, these compounds exhibit comparable distribution concentrations within blood plasma, lack cytochrome P450 (CYP) substrate and inhibitory properties, exhibit enhanced rates of excretion from the body, and possess a toxicity level that is deemed safer than Isoniazid. Specifically, these compounds demonstrate non-hepatotoxicity, as evidenced by LD50 values of 1350 m/kg (FA) and 290 m/kg (BA). Furthermore, utilizing in-silico molecular docking techniques, it was determined that Fumaric Acid (FA = -4.8 kcal/mol) exhibits a higher binding affinity compared to Benzoic Acid (BA = -5.4

kcal/mol) towards acidic residues. A higher number of amino acids suggests a favourable interaction between the ligand and the 4KL9 receptor in a legitimate conformation, as evidenced by the root-mean-square deviation (RSMD) binding affinity value falling within the range of 0-12. The Ranggap banana plant, which contains significant amounts of fumaric acid (FA) and benzoic acid (BA), shows great potential as a therapeutic treatment for persons suffering from tuberculosis. Notably, its medicinal properties surpass those of manufactured pharmaceuticals, including the antibiotic Isoniazid. In addition to future prospects, there is an expectation that pharmaceutical professionals would undertake further analysis of the potential of various substances derived from Ranggap bananas in the development of herbal treatments for tuberculosis.

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