# *In silico* study of phytochemicals contained in *Brucea javanica* in inhibiting the InhA enzyme as antituberculosis

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## Abstract

**Background**: Currently *Mycobacterium tuberculosis* is found to be resistant to the treatment of tuberculosis with rifampin and isoniazid (INH) and often stated as multi-drug resistance (MDR). Knowledge and determination of biological properties of plant extracts is a source of drug candidates in various health fields. Therefore, natural products are important in the discovery of new drugs, especially in disease therapy, particularly for tropical diseases, tuberculosis. *Brucea javanica,* known as *Buah Makasar*, is found in many Asian countries including Indonesia. This plant fruit has a very bitter taste so it cannot be directly consumed and is often used as a traditional medicine to prevent some diseases, especially malaria. There has been no research on the effectiveness of *Buah Makasar* in tuberculosis.

**Objective**: This study aims to identify compounds contained in *Brucea javanica*, namely *bruceines, bruceosides* and *yadanziosides* in inhibiting the InhA enzyme found in the wall of *Mycobacterium tuberculosis*.

**Methods**: This *in-silico* study is using Molegro Virtual Docker (MVD) Ver. 5.5. We compared it to the native ligand, namely *N*-(4-Methylbenzoyl)-4-Benzylpiperidine (code: 4PI) and the reference drug standard, INH.

**Results**: *In-silico* results show that *yadanziosides* found in *Brucea javanica* have the potential to inhibit the InhA enzyme. Bruceoside F (-190.76 Kcal/mol) has the lowest MolDock score among the 27 other compounds. It is also having lower MolDock score than the native ligand 4PI (-120.61 Kcal/mol) and INH (-54.44 Kcal/mol).

**Conclusion**: *Brucea javanica* can be considered as source of drug development for againts tuberculosis.

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Key words: Derivatives of brucein, Bruceocide and yadanzioside, Molecular docking, Tuberculosis, *Brucea javanica*, Prediction.

Acknowledgments: The authors are grateful for research funding from Universitas Airlangga (PUF scheme) year of 2021.

Contributions: MIS, GSP, substantial contributions to the conception or design of the work; TW, the acquisition, analysis, and interpretation of data for the work; KM, JE, drafting the work or revising it critically for important intellectual content. All author approved the final version to be published.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Availability of data and material: Data and materials are available by the authors.

Informed consent: The manuscript does not contain any individual person's data in any form.

Received for publication: 13 November 2022.
Revision received: 5 January 2023.
Accepted for publication: 9 January 2023.



*Journal of Public Health in Africa 2023; 14(s1):2518*
*doi:10.4081/jphia.2023.2518*

## Introduction

Lower respiratory infections have been one of the 10 deadliest diseases in the last decade. There are several types of these diseases such as pneumonia, bronchitis and tubercolosis (TB) among the top 10 deadly diseases in the world. According to WHO data in 2019, lower respiratory tract infections account for about 5.7% of deaths in the world or about 3.2 billion people have died.[1] One of the lower respiratory tract infectious diseases of concern to researchers is TB infection. Indonesia as the developing country with the most population still faces this infection problem.[2] TB infectious disease is caused by the bacteria *M. tuberculosis*.

Currently *M. tuberculosis* is found to be resistant to the treatment of tuberculosis with rifampin and isoniazid (INH) or which is often stated as multi-drug resistance (MDR). Many cases of MDR are encountered during treatment using rifampin and INH as the primary therapy. This is what causes the indispensability of new anti-TB drug candidates. Therefore, we need to conduct a research in an effort to find TB drugs that are more active and effective in overcoming MDR problems against *M. tuberculosis*.[3]

Natural ingredients have been known for a long time as the main source of drug development because of the diverse and complicated structure of them. Often, such organic compounds from nature have high biological activity. As it is known that only about 100 small molecules were accepted as new drugs for decades, in which about 50% of them were of natural origin. Therefore, natural products and derivatives are still useful in the discovery of new drug compounds.[4,5]

One of so many ways to produce new drug is by doing chemical modification. Since 2010, a new approach has been introduced through chemical modifications of plant extracts to produce new active compounds. This approach is a method to alter the-chemical components in plant extracts, and will affect their biological activity. There are some methods of chemical modifications of plant extracts.[6-8]

Indonesia is the country with the greatest biodiversity in the world, which has 17500 islands and more than 100 million hectares of protected forest. In addition, Indonesia also has 30,000 medicinal plants that have biological activities (approximately 6000 plants). In traditional medicine, there are 100 medicinal plants that are often used by the Indonesian people, which are referred to as herbs. Traditional Indonesian medicine consists of a single plant material or a mixture of several medicinal plants, which are used both in disease prevention and in treating certain diseases for generations.[9]

The use of medicinal plants for a better life, reduced disease and improved quality of life has been proven. Natural ingredients have been believed to be the greatest source of drug discovery and development due to the uniqueness and complexity of their structures. The uniqueness of the structure of compounds in natural materials has a role to play in their biological activity. Several studies describe the use of traditional crops over the years and are important in the pharmaceutical industry. Knowledge and determination of biological properties of plant extracts is a source of drug candidates in various health fields. Therefore, natural ingredients are important in the discovery of new drugs, especially in disease management, particularly tropical diseases, one of which is TB.

*Brucea javanica,* which is known as *Makasar Fruit,* is found in many asian countries including Indonesia. This plant takes the form of an upright shrub every year and grows wild in the forest or yard. This plant fruit has a very bitter taste so it cannot be directly consumed and is often used as a traditional medicine to prevent several diseases, especially malaria.[10] From literature studies, there has been no research on the effectiveness of *Makasar Fruit* in TB disease. The urgency of this study is the importance of knowing the ability of *Makasar Fruit* in inhibiting *M. tuberculosis* bacteria *in silico*. This research is also based on the problem of the lack of usefulness of indigenous Indonesian herbal medicines in disease management, especially tropical diseases, such as TB.

Phytochemicals contained in *Brucea javanica* have been identified through chemical structure elucidation. Major Phytochemicals contained in *Brucea javanica* are quasinoid compounds that are terpenoid derivatives.[11] The quasinoid compounds found in *Brucea javanica* can be classified into three groups based on their chemical structure, namely Bruceines, Bruceosides and yadanziosides that will be tethered to the InhA enzyme.

Mycobacterium enoyl acyl carrier protein reductase (InhA) is a key target for antitubercular drug discovery. InhA enzymeis an enzyme, which catalyzes the reduction of long-chain trans-2-enoyl-ACP in type II fatty acid synthesis (FAS II) for meromycolic acids pathway of Mycobacterium tuberculosis.[12,13] Meromycolic acids are bio-starting material for mycolic acids, which are essential and major components of mycobacterial cell walls (Figure 1).[12]

Some studies targeted InhA enzyme to develop anti-TB because InHA enzyme is easily to have resistance to TB treatment.[12,14,15] If the resistance is happened, the walls of Mycobacterium tuberculosis will become thicker with mycolic acids so that first-line TB therapies such as Rifampicin, Rifanbutin and Rifapentine which inhibit RNA polymerase cannot penetrate properly into the cytosol of Mycobacterium tuberculosis. Moreover, second-line and third-line TB therapy drugs, such as the antibiotic oxazine class (Levofloxacine, Moxifloxacin,

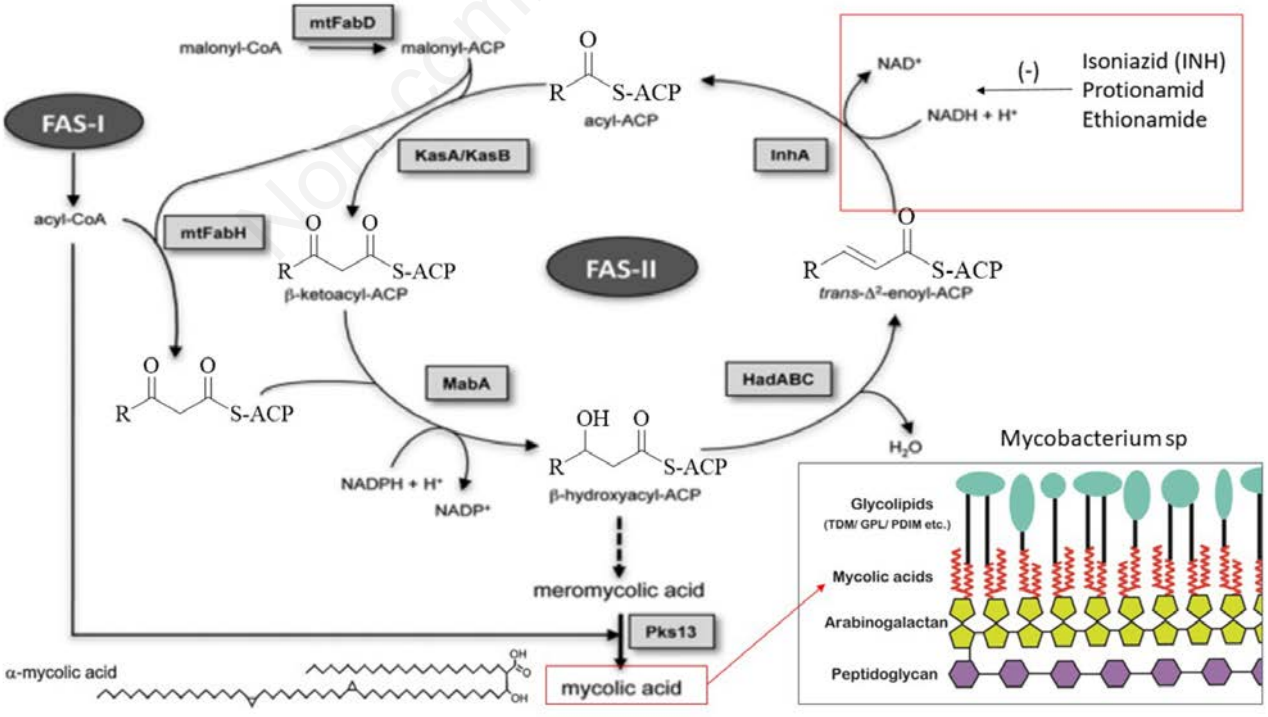



**Figure 1. Biosynthesis of mycolic acid in *mycobacterium tuberculosis*.[12]**

Gatifloxazin) with DNA gyrase inhibitor mechanisms will not work effectively to penetrate the cytosol of *Mycobacterium tuberculosis* (Figure 2). As only aminoglycoside class antibiotics such as (Streptomycin, Kanamycin, Amikacin) also won't work effectively to penetrate into the cytosol of *Mycobacterium tuberculosis,* which inhibits protein synthesis in the ribosome (Figure 2). Therefore, the pattern of drug discovery and development in anti-TB drugs focuses a lot on cell wall synthesis through Mycolic acid inhibitors by inhibiting InhA enzyme (Figure 2).[12,14,15]

## Materials and Methods

The tools used are AMD A6 Vision laptop with CPU specification @ 1.4 GHz, 4 GB of RAM, and Chembiodraw version 11, and Molegro Virtual Docker (MVD) Ver.5.5. MVD software is one of the paid programs used for molecular docking studies with the match results accuracy reaching above 90%.[16] InhA enzyme is downloaded from the protein data bank (http://www. rcsb. org/-pdb) with PDB ID: 2NSD at 1.9 Å resolution.[13,14] Phytochemicals from *Brucea javanica* are bruceines, bruceosides and yadanziosides (Figure 3) that have been identified and well documented on www.pubchem.com and several scientific journal publications.[16,17]

Preparation of ligands of *bruceines, bruceosides* and *yadanziosides* were performed by drawing the 2D ligands in Chembiodraw. Then, it is converted to the 3D ligands. We calculated the minimal energy of the 3D ligands, where known as the most stable form by applying MMFF94 calculation and the ligands are stored in the Protein Data Bank file type.[18]

The receptors were obtained from PDB that is prepared with MVD. InhA enzyme and its comparative ligand, namely N-(4-Methylbenzoyl) -4-Benzylpiperidine (code: 4PI) and the isoniazid *standard drug reference* (code: INH). A re-docking process was carried out to validate MVD.[12,19] This is conducted in order for it being able to be used in redocking the phytochemicals compounds from *Brucea javanica.* The redocking acceptance parameter is the value of Root Mean Square Deviation (RMSD) <2.0 Å.

The docking result obtained is the MolDock score, which is interpreted as a prediction of the bond interaction between the drug and the receptor. It is the total energy from external ligand interaction plus internal ligand interaction (Figure 4). The smaller the MolDock score, the greater the degree of compatibility between the ligand and the receptor to interact. The docking results can also be visualized and interpreted to provide a picture of the interaction of ligand bonds with receptors, which includes hydrogen bond interactions, hydrophobic interactions and electronic interactions.

## Results

The RMSD value shows the conformity of the ligand coordinates from the crystallographic results compared to the ligand coordinates that are re-docked by 4PI in InhA enzyme of 0.45 Å in accordance with the docking process criteria (Figure 5).

The docking data of *Phytochemicals* compounds from *Brucea javanica* using MVD was presented in Table 1.

## Discussion

Compounds that have a docking value lower than the native ligand 4PI (-120.61 Kcal/mol) are five compounds of *bruceines derivatives* (bruceine A; bruceine B; bruceine C; bruceine J, bruceintiol); six compounds of *bruceosides derivatives;* and nine compounds of *yadanziosides derivatives* (yadanzioside A; yadanzioside B; yadanzioside; yadanzioside D; yadanzioside E; yadanzioside F; yadanzioside G; yadanzioside L'yadanzioside M; yadanzioside P). These results are presented in Table 1. *Bruceines derivatives* as much as 50% have a lower docking value than the native ligand 4PI, while for *bruceosides derivatives*, it is as much as 100% and for *yadanziosides derivatives*, it is as much as 90%.

The compound with the lowest MolDock score is bruceoside

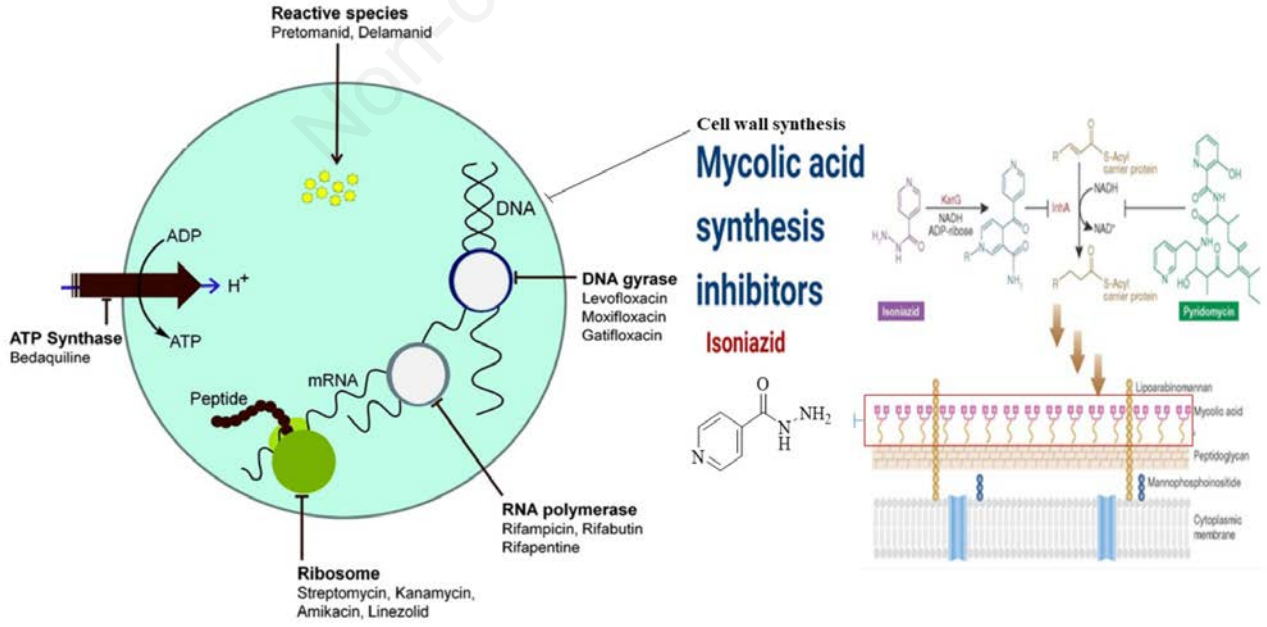



**Figure 2. The mechanism of some anti-TB medicines.**

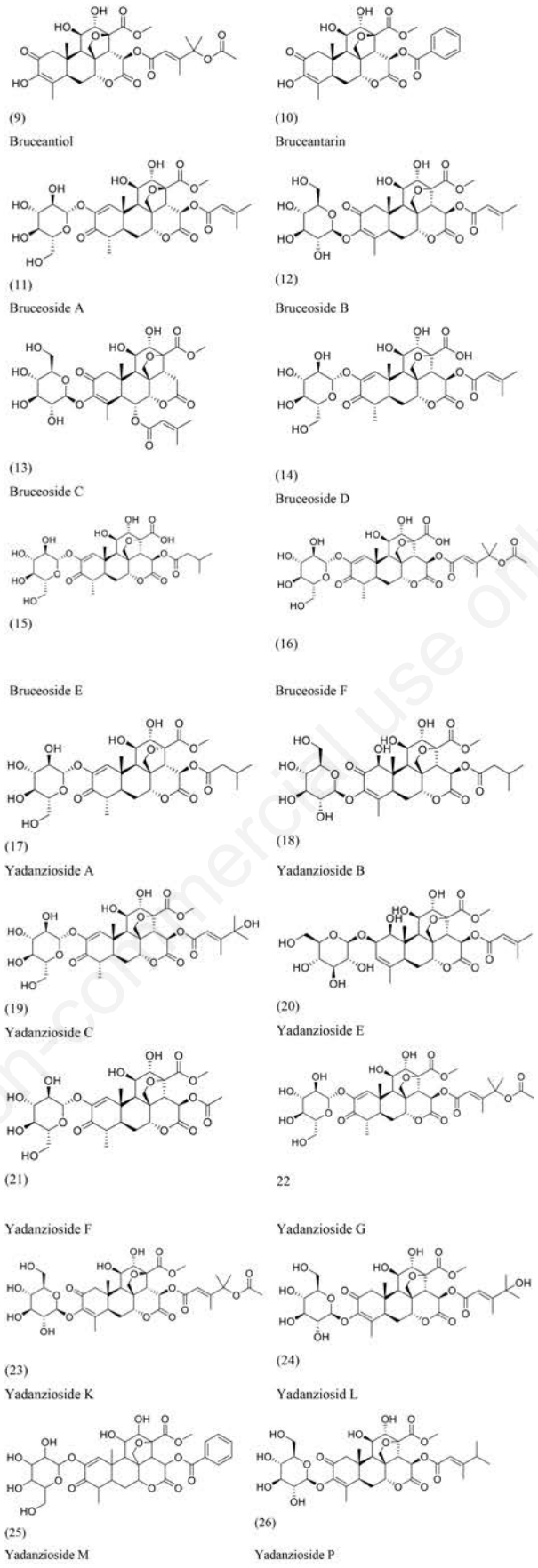





**Figure 3. Phytochemicals in *Brucea javanica* docked to InhA enzyme.**

F[17] with a MolDock score of -190.76 Kcal/mol which is a *bruceosides derivatives* compound. Bruceoside F not only has the lowest MolDock score but also has the most hydrogen bond interactions in InhA enzyme with a hydrogen bond value of –11.83 Kcal/mol. Bruceoside F is predicted to inhibit InhA enzyme directly better than its native ligand, N-(4-Methylbenzoyl)-4-Benzylpiperidine (4PI). In contrast, INH has a poor docking score against InhA protein because isoniazid does inhibit InhA enzyme indirectly by being activated by Kat G (catalase peroxidase) which oxidizes INH to an acyl radical binding at position 4 of Nicotinamide Adenine Dinucleotide (NAD), forming INH-NAD additions (Figure 2).

Among of all the quasinoid derivatives found in *Brucea javanica*, *bruceosides derivatives* are predicted to be the most powerful in inhibiting InhA enzyme directly produced by *Mycobacterium tuberculosis* because bruceoside F compounds[17] has the lowest

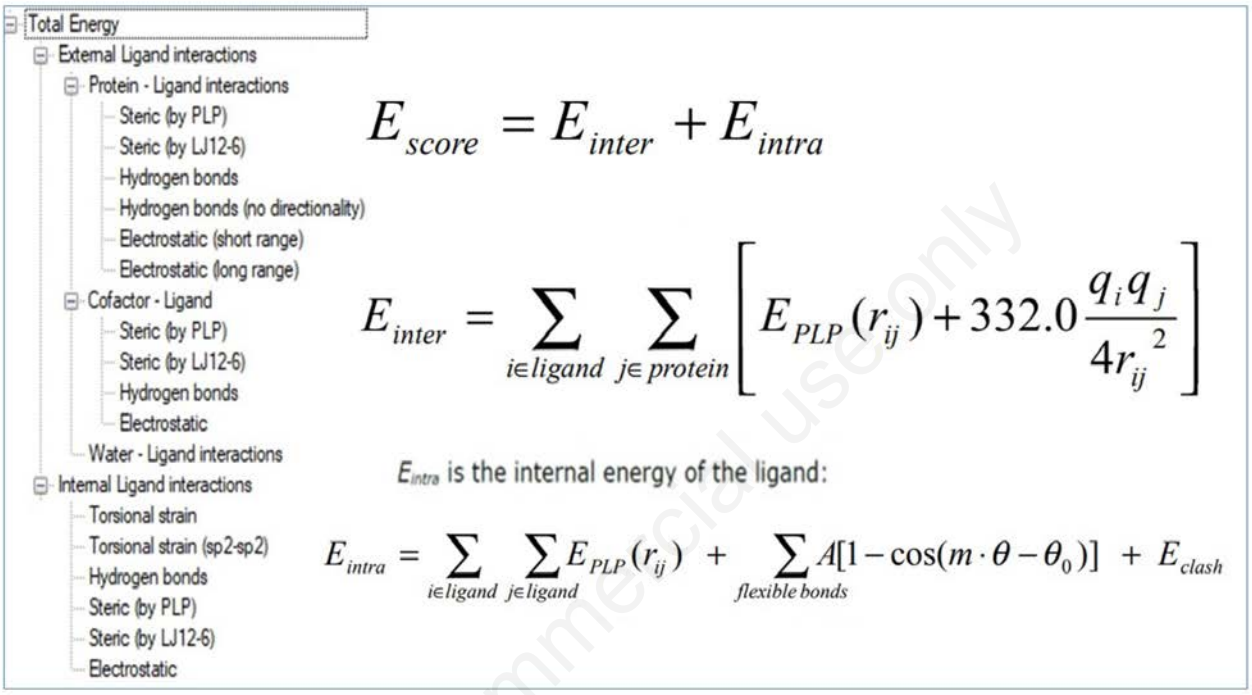



**Figure 4. The formula for getting moldock score by MVD.**

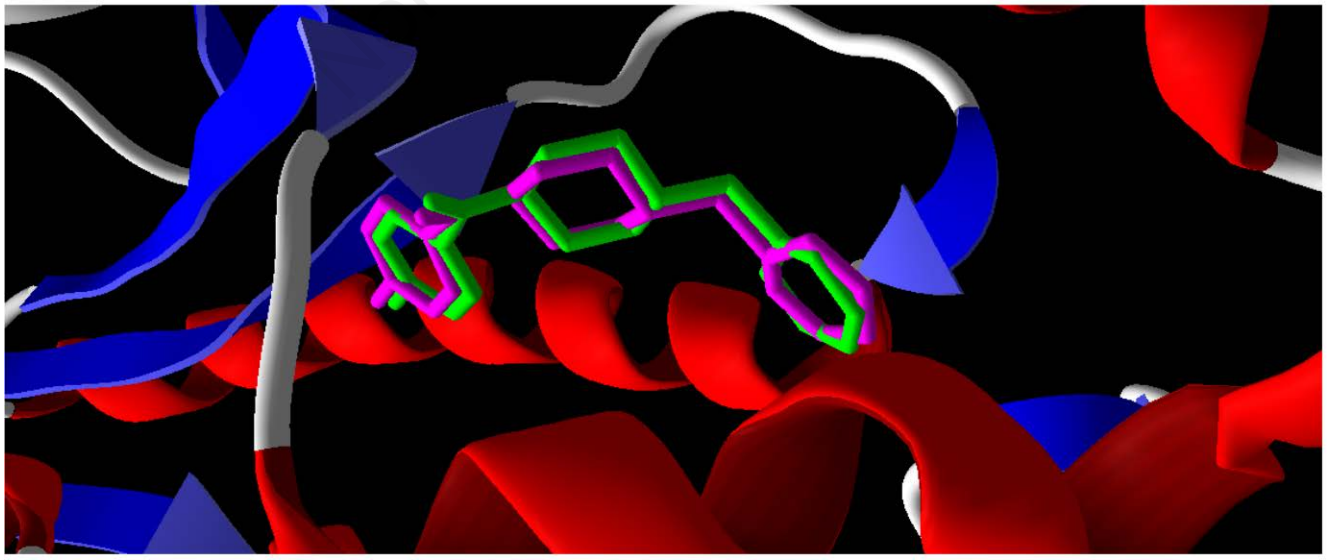
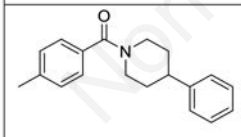
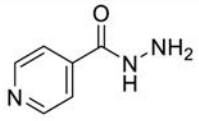
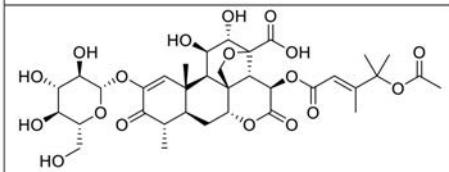


**Figure 5. Visualization of the *4PI* redocking process on InhA enzyme with RMSD 0.45 Å.**

**Table 1. Results of MolDock score of phytochemicals contained in brucea javanica, the native ligand and the standard drug.**

| No | Phytochemicals | Moldock score (Kcal/mol) | Hydrogen bond binding energy (Kcal/mol) | Docking posed |
|---|---|---|---|---|
| 1 | Bruceine A | -143.34 | 0.78 | ✓ |
| 2 | Bruceine B | -121.92 | 4.37 | ✓ |
| 3 | Bruceine C | -150.77 | 3.38 | ✓ |
| 4 | Bruceine D | -88.60 | 0 | ✓ |
| 5 | Bruceine E | -84.27 | 1.43 | ✓ |
| 6 | Bruceine H | -90.07 | 3.02 | ✓ |
| 7 | Bruceine I | -103.67 | 2.05 | ✓ |
| 8 | Bruceine J | -136.76 | 1.43 | ✓ |
| 9 | Bruceantiol | -154.74 | -2.5 | ✓ |
| 10 | Bruceantarin | -90.77 | 6.65 | ✓ |
| 11 | Bruceoside A | -150.61 | 11.63 | ✓ |
| 12 | Bruceoside B | -167.68 | 3.38 | ✓ |
| 13 | Bruceoside C | -160.80 | -4.82 | ✓ |
| 14 | Bruceoside D | -149.86 | -11.77 | ✓ |
| 15 | Bruceoside E | -151.98 | 9.34 | ✓ |
| 16 | Bruceoside F | -190.76 | -11.83 | ✓ |
| 17 | Yadanzioside A | -147.51 | -9.08 | ✓ |
| 18 | Yadanzioside B | -158.41 | 9.40 | ✓ |
| 19 | Yadanzioside C | -177.11 | -8.62 | ✓ |
| 20 | Yadanzioside E | -154.11 | -9.36 | ✓ |
| 21 | Yadanzioside F | -135.15 | 9.03 | ✓ |
| 22 | Yadanzioside G | -187.17 | -9.55 | ✓ |
| 23 | Yadanzioside K | -116.45 | 5.44 | ✓ |
| 24 | Yadanzioside L | -155.35 | 10.13. | ✓ |
| 25 | Yadanzioside M | -181.08 | -11.71 | ✓ |
| 26 | Yadanzioside P | -160.28 | 6.22 | ✓ |
| 27 | 4PI | -120.61 | 0.19 | ✓ |
| 28 | INH | -54.44 | 2.64 | ✓ |

**Table 2. Data on the interaction of amino acids of 4PI, INH and bruceoside F compounds in InhA enzyme.**

| Compound | Hydrogen bond | Residues involved | Steric interaction | Residues involved |
|---|---|---|---|---|
|  4PI | - | - | 4 | Gly 104<br>Tyr 158<br>Gly 192<br>Ile 202 |
|  INH | 2 | Gly 192<br>Ile 194 | - | - |
|  bruceoside F | 7 | Gly 14<br>Ser 20<br>Ser 94<br>Gly 96<br>Ile 194<br>Thr 196<br>Met 199 | 8 | Ile 16<br>Ser 20<br>Ile 21<br>Ser 94<br>Met 147<br>Tyr 158<br>Met 199<br>Trp 222 |

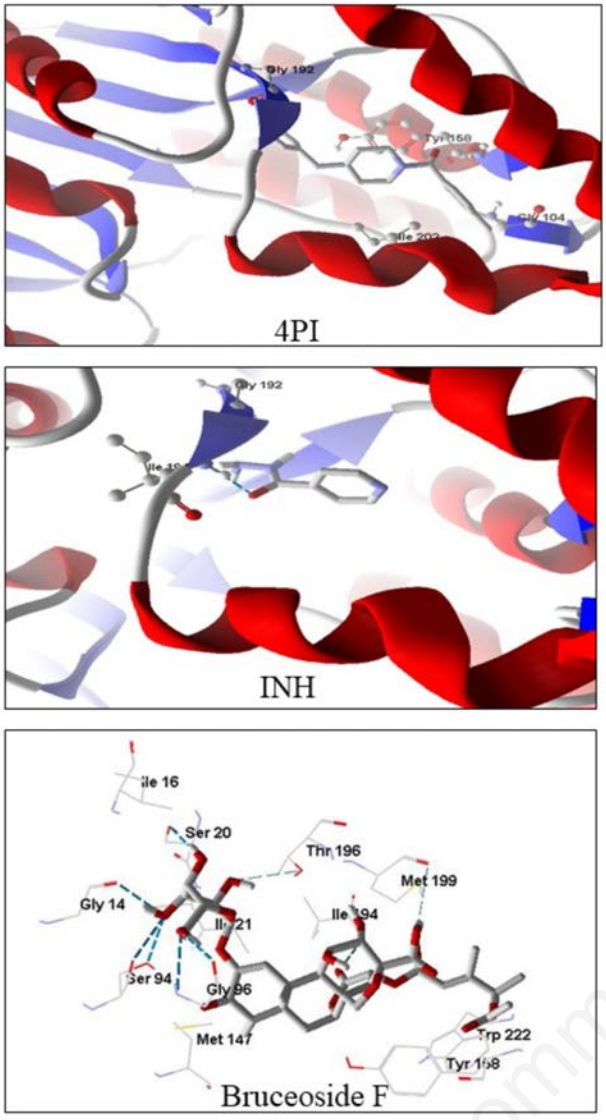



**Figure 6. Hydrogen bond interaction (blue line) and steric interaction of 4PI, INH and bruceoside F compounds on InhA enzyme.**

MolDock score compared to the native ligands and other compounds. Hydrogen bond interaction (blue line) and steric interaction (red line) of 4PI, INH and bruceoside F compounds on InhA enzyme can be seen in Figure 6 and Table 2.

Hydrogen bond interactions that are only found in INH and bruceoside F compounds in 4PI compounds which are native ligands are not found because 4PI compounds do not have donor hydrogen atoms and only have two hydrogen atom acceptors. INH compounds have respectively two hydrogen atom donors and acceptors, so that the hydrogen bond interactions in InhA protein of amino acids Gly 192 and Ile 194 are possible. Bruceoside F has a hydrogen donor and an asymmetric acceptor in excess of the glycone group attached to bruceoside F which has an impact on the number of hydrogen bond interactions in the InhA protein of amino acids Gly 14; Ser 20; Ser 94; Gly 96; Ile 194; Thr 196; Met 199. In some of those hydrogen bond interactions, we also found the acarbose of amino acids Asp 69; Arg 213; Asp 352; Arg 442. Moreover, bruceoside F is also found in some of the hydrogen bond interactions in INH.

Bruceoside F has the best binding energy among other compounds so that it has a low impact on the MolDock score when it docked in InhA enzyme. Some compounds that also show good hydrogen energy binding (< -10Kcal/mol) are represented by bruceoside compounds A (-11.63 Kcal/mol) and D (-11.77 Kcal/mol) and *yadanziosides derivatives*, namely yadanzioside L (-10.13 Kcal/mol) and M (-11.71 Kcal/mol). The most influential hydrogen bond interaction giving the lowest bond energy is Gly 96 (-4.92 Kcal/mol) with a bond distance of 3.0 Å as shown in Figure 2. The steric interaction of bruceoside F compound is very complex compared to 4PI and INH of 8 amino acids Ile 16; Ser 20; Ile 21; Ser 94; Met 147; Tyr 158; Met 199; Trp 222.

## Conclusions

Bruceoside F is a *bruceoside derivative* contained in which is strongly expressed to inhibit InhA enzyme directly better than the native ligand 4PI and INH (isoniazid) as an antituberculosis drug which needs to be tested *in-vitro* on *Mycobacterium tuberculosis* in the future study.



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