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RESEARCH ARTICLE

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Phytofluene from *Physalis peruviana* as promising anti-TB via InhA of *Mycobacterium tuberculosis* target: an *in silico* research

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Abstract

Tuberculosis (TB) is an infectious disease after severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) in developed countries including Indonesia. Drug resistance becomes major issue worldwide and needs prospective therapeutics development. Plant with medicinal properties including *Physalis peruviana* is one the promising object to be new anti-TB drug candidate. This study aimed to analyze the inhibitory activity of anti-TB agents from aerial parts of *P. peruviana*. Ligand and protein samples were obtained from PubChem and RCSB PDB, respectively. The bioactive compounds were evaluated their antibacterial prediction and drug-likeness properties throughout PASS Online and SwissADME webservers. Selected ligands then docked via PyRX and measured the output by binding affinity. Visualization of the best outputs was carried out using BIOVIA Discovery Studio. The result showed that phytofluene had the lowest binding affinity topping the isoniazid as control with -7.2 and -5.1 kcal/mol after targeting enoyl-[acyl-carrier-protein] reductase (InhA) protein of Mycobacterium tuberculosis. This concluded that phytofluene functioned as predictive anti-TB therapeutic candidate. Further *in vitro* and *in vivo* studies are needed to validate this outcome.

Keywords: Phytofluen, *Physalis peruviana*, Tuberculosis, InhA, Antibacterial

Introduction

Tuberculosis (TB) is a communicable infectious disease and the leading cause of illness specifically in developed countries. This disease is caused by the opportunistic bacillus *Mycobacterium tuberculosis* (MTB) which initially targets the lungs and may later spreads to the other part of the body[1].

According to the World Health Organization (WHO) database, a total of 1.3 million people died from TB in 2022, making it the second greatest infectious killer after coronavirus disease 2019 (COVID-19)[2]. Human immunodeficiency virus (HIV)-infected patients accounted 0.013% of the total mortality in the world because they developed 19 times susceptibility to TB[1,2]. Indonesia with other 29 countries became the highest number cases of new 86% of TB worldly in 2020[3].

Several medications have been authorized by the United States Food and Drugs Administration (US-FDA) to improve TB. Isoniazid, together with rifampin, ethambutol, and pyrazinamide, was the first-line of TB treatment that administered daily for several months[4]. Isoniazid has mechanism actively triggered by the catalase-peroxidase enzyme (KatG) later inhibits mycolic acid formation for cell wall construction. However, TB resistances produced several mutations mainly in enoyl-[acyl-carrier-protein] reductase (InhA) genes and leading to isoniazid resistance therapy[5]. Other medicines have been discovered, yet the resistances persist in numerous countries.

In Indonesia, several weeds were recognized to have therapeutic benefits[6]. *Physalis peruviana* is one of the herbaceous plants producing rich phytochemicals throughout the body. However, the consumption of this plant is restricted on the fruits, which are traditionally applied to cure stomachache and smallpox among Karo sub-ethnic in North Sumatra[7]. Based on the various articles, the aerial parts of *P. peruviana* contained high concentration of phytoconstituents[8,9]. However, no records found of *P. peruviana* phytocompounds treated as anti-TB constituent before. These chemicals can be utilized to be simulated as a drug in various *in silico* technics. Thus, the objective of this study was to analyze the inhibitory activity of anti-TB agents derived from *P. peruviana* aerial elements.

Materials and methods

Study area

This study conducted using *P. peruviana* bioactive compounds from the aerial portions and target protein were mined from online database[9,10]. Canonical SMILES of the compounds were obtained to identify the antibacterial potential and drug-likeness in public webservers. Selected phytochemicals would be selective docked to *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA) and evaluated the chemical interactions through different softwares.

Procedures

Ligand and Protein Preparation

The compounds contained in upper body of *P. peruviana* and control ligand isoniazid (CID 3767) were retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/). The information obtained from this database consisted of CID, 3D structure, and canonical SMILES. Optimization was required to reduce the compound energy and saved in PDB format for molecular docking analysis utilized PyRx 0.9.9 software[11]. In addition, InhA protein was downloaded in PDB file from RCSB PDB (https://www.rcsb.org/). Protein was sterilized to remove the water, native ligand, and other compartment contents for screening purpose through BIOVIA Discovery Studio 2020 software[12].

Bioactivity Prediction and Drug-likeness Identification

Selected compounds obtained from the database were screened using PASS Online webserver (https://www.way2drug.com/passonline/) to determine the antibacterial inhibition. Threshold of this prediction was set higher than 0.3 for the predictive activity (Pa >0.3)[13]. Also, drug-likeness identification was conducted using the SwissADME webserver (https://www.swissadme.ch/) to identify constituents that might have medicinal qualities that intergrated with those of the Lipinski rules. Filtered drug candidates would be reviewed by fulfilling molecular weight (≤500 Da), high lipopolicity (≤5), hydrogen bond donor (HBD, ≤5), hydrogen bond acceptor (HBA, ≤10), and molar refractivity (40-130)[14].

Molecular Docking Simulation

Molecular docking simulation method is part of *in silico* approach for determining the inhibitory ability of the ligands to the target protein. This method employed was specific docking that focused on the active sites of Myc inhA and carried out using PyRx 0.9.9 version with academic license[10,13,15].

Data analysis

Docking results were downloaded per interaction and analyzed throughout the binding affinity (kcal/mol) and chemical bonds. The position and interaction of docking analysis were developed by BIOVIA Discovery Studio. The best results of structure of ligand-protein interactions visualized in 3D and 2D structures consisted specific staining and interactions[14].

Results

Bioactive Compounds in Aerial Parts of* P. peruviana *and* InhA *of* M. tuberculosis *Based on Database

Based on the literature review, there were 15 phytochemicals found in aerial body of P. peruviana. Those compounds were assessed in PubChem to get the CID, 3D structure, and canonical SMILE. However, only 11 compounds left on the PubChem alongside with isoniazid[9]. On the other hand, InhA protein complex was retrieved with several ligands and supplemented with active sites information from related sources[10,15] (**Table 1 and 2**).

Table 1. Bioactive Compounds from Aerial Parts of *P. peruviana*.

Bioactive Compounds	CID	Canonical SMILE
3α-Tigloylnxytropane	-	-
3β-Acetoxytropane	-	-
Antheraxanthin	5281223	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC23C(CC(CC2(O3)C)O)(C)C)C)C
Cuscohygrine	1201543	CN1CCCC1CC(=O)CC2CCCN2C
Hygrine	440933	CC(=O)CC1CCCN1C
Lutein	5281243	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CC(CC2(C)C)O)C)C)C
Neoxanthin	5282217	CC(=CC=CC=C(C)C=CC=C(C)C=C=C1C(CC(CC1(C)O)O)(C)C)C=CC=C(C)C=CC23C(CC(CC2(O3)C)O)(C)C
N-Methylpyrrolidinylhygrine A	-	-
N-Methylpyrrolidinylhygrine B	-	-
Physoperuvine	443008	CN1C2CCCC1(CC2)O
Phytofluene	6436722	CC(=CCCC(=CCCC(=CCCC(=CC=CC=C(C)C=CC=C(C)CCC=C(C)C)C)C)C)C
Tropine	449293	CN1C2CCC1CC(C2)O
Violaxanthin	448438	CC(=CC=CC=C(C)C=CC=C(C)C=CC12C(CC(CC1(O2)C)O)(C)C)C=CC=C(C)C=CC34C(CC(CC3(O4)C)O)(C)C
Zeaxanthin	5280899	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CC(CC2(C)C)O)C)C)C
Gamma-Carotene	5280791	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC=C(C)CCC=C(C)C)C)C-

Table 2. InhA protein of *M. tuberculosis*.

Protein	PDB ID	Active Site
InhA	7E48	Phe149, Tyr158, Thr196, and Ala198

Bioactivity Prediction and Drug-likeness Identification

In this screening, compounds with a Pa value of greater than 0.3 were chosen. A Pa value of more than 0.3 suggests the constituents had potential for carrying out related antibacterial. In addition, drug-likeness identification selects phytochemicals that have drug-like characteristics. Six compounds were filtered and fulfilled the bioactivity prediction and drug-likeness identification (**Table 3**).

Table 3. Bioactivity Prediction and Drug-likeness Identification of Selected Bioactive Compounds.

Bioactive Compounds	Pa	MW	HBD	HBA	MR	LogP	Status
Antheraxanthin	0.290	584.87	2	3	186.26	8.82	×
Cuscohygrine	-	224.34	0	3	74.20	1.59	×
Hygrine	0.179	141.21	0	2	45.4	1.06	×
Lutein	0.531	568.87	2	2	186.76	10.40	√
Neoxanthin	0.302	600.87	3	4	73.22	8.72	√
Physoperuvine	0.255	141.21	1	2	44.35	1.12	×
Phytofluene	0.439	542.92	0	0	189.65	13.61	√
Tropine	0.295	141.21	1	2	44.31	0.86	×
Violaxanthin	0.358	600.87	2	4	185.80	8.97	√
Zeaxanthin	0.436	568.87	2	2	186.76	10.55	√
Gamma-Carotene	0.383	536.87	0	0	186.33	12.77	√

Molecular Docking Simulation and Visualization

After filtering in previous steps, we got 5 bioactive compounds that matched antibacterial prediction and drug-likeness parameters such as lutein, neoxanthin, violaxanthin, zeaxanthin, and gamma-caroten. Those ligands would be simulated by computer with isoniazid as control. Molecular docking was carried out by specific docking and resulted phytofluene as the most effective inhibitors than the control. In contrast, other constituents had positive binding affinity outcomes that indicated the ineffective inhibition (**Table 4 and 5**).

Phytofluene was chosen to determine the chemical interactions and visualization with comparison by the isoniazid. According to the chemical interaction, phytofluene formed strong hydrophobic interaction category with pi-sigma, alkyl, and pi-alkyl types against several active sites. In contrast, isoniazid had only few interactions with InhA of *M. tuberculosis* by hydrogen bonds and hydrophobic interaction. These findings revealed no active site interaction (**Table 5, Figure 1**).

Table 4. Molecular Docking Results of Selected Bioactive Compounds of *P. peruviana* against InhA of *M. tuberculosis*.

Center (Å)	Dimension (Å)	Bioactive Compounds	Binding affinity (kcal/mol)
X: 62.295 Y: -8.551 Z: 39.093	X: 12.950 Y: 19.990 Z: 16.059	Lutein	181.7
		Neoxanthin	34.5
		Phytofluene	-7.2
		Violaxanthin	136.4
		Zeaxanthin	227.4
		Gamma-Carotene	97.1
		Isoniazid (Control)	-5.1

Table 5. Chemical Interaction of Docking Outcomes.

Ligand	Residues	Interaction	
		Category	**Type**
Phytofluene	**Phe149**	Hydrophobic Interaction	Pi-Sigma
	Ile16, Ile21, Ile21, Ile21, Met147, Met147, Met161, Met161, Met161, Lys165, Ala191, Ile194, **Ala198**, **Ala198**, Met199, Met199, Ile202, Ile202,		Alkyl
	Phe149, **Tyr158**, **Tyr158**		Pi-Alkyl
Isoniazid	Gly14, Ile21, Ser94, Ser94	Hydrogen Bond	Conventional
	Ile21	Hydrophobic Interaction	Pi-Sigma

Note: Active sites written in bold.

Discussion

In silico approach to analyze antibacterial activity of *P. peruviana* metabolites from aerial body targeted with InhA of M. tuberculosis revealed its inhibitory activity and pharmacological characteristics from phytofluene. This compound was detected diversely in *P. peruviana* parts like in aerial parts, fruits, and pulps and mainly extracted with hexane/acetone/ethanol[8,16]. Phytofluene altogether with phytoene are colorless carotenoids that serve as a source of vitamin A precursors and promote health in human body through antioxidative, anti-proliferative, and anti-inflammatory activities[17–19]. Colorless-caratenoid containing foods are thought have many benefits including a reduction in cardiovascular-associated, cancer, and UV-light induced erythema diseases[20–22].

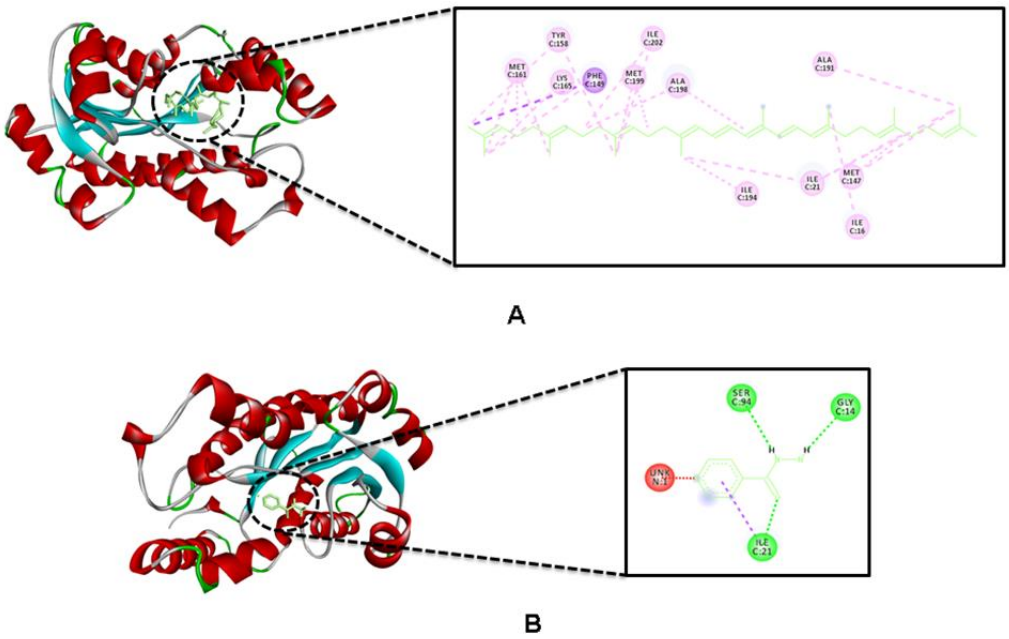

Figure 1. InhA of M. tuberculosis interaction after molecular docking against: A) phytofluene from *P. peruviana* and B) isoniazid.

InhA is a prime candidate for drug design to combat drug resistant during TB[10]. This protein catalyzes the NADH-dependent reduction of enoyl-ACP in the fatty and mycolic acid biosynthesis,

which form vital component of the membrane and cell wall of M. tuberculosis, respectively. Tyr158 and Thr196 are two conserved active site residues that involved in protonation and hybrid transfer during the catalysis[23]. As a result, this protein is critical for development of future anti-TB therapies[24].

Based on the molecular docking, phytofluene had the most negative results, which means the most effective inhibitory prediction against InhA. This finding outperformed isoniazid as first-line drug of common TB treatment which used in decades around the world. Despite the lack of hydrogen bonds, hydrophobic interactions of phytofluene were diversified and localized to a number of active sites (Phe149, Tyr158, and Ala198) (**Table 5, Figure 1**). Hydrophobic interactions play vitally in biological and cellular activities, resulting stability and turnover of proposed interaction[25].

Conclusions

Phytofluene from *P. peruviana* exhibits promising potential as anti-TB agents due to their capacity to inhibit the interaction of InhA, surpassing the isoniazid as control. This outcome needs additional trials with *in vitro* and *in vivo* analyses to strengthen the evidence of *in silico* study.

Acknowledgments

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Conflicts of Interest

There are not potential conflicts of interest.

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