

Original Research Article

In silico anti-tuberculosis and immunomodulatory effect of mitragynine

Ari Nuswantoro^{1,2}, Jusak Nugraha³, Sri Agus Sudjarwo⁴, Purwo Sri Rejeki⁵,
Dinasti Aprillia⁶

¹Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, ²Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak, Pontianak, ³Department of Clinical Pathology, Dr. Soetomo General Academic Hospital, ⁴Department of Pharmacology, Faculty of Veterinary Medicine, ⁵Physiology Division, Department of Medical Physiology and Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, ⁶Unit Pelaksana Teknis Pusat Laboratorium Kesehatan Kota Pontianak, Pontianak, Indonesia

*For correspondence: **Email:** jusak-n@fk.unair.ac.id; **Tel:** +628123183786

Sent for review: 23 September 2025

Revised accepted: 20 February 2026

Abstract

Purpose: To investigate the dual potential of mitragynine as an anti-tuberculosis and immunomodulatory agent by *in silico* evaluation of its pharmacokinetic properties and binding affinities for *Mycobacterium tuberculosis* (Mtb) proteins and pro-inflammatory cytokines.

Methods: Mitragynine and 7-hydroxymitragynine, the two most abundant alkaloids in kratom leaves, were evaluated for drug likeness and synthetic accessibility. Molecular docking was performed against four Mtb-related proteins (isocitrate lyase, RmlD, BioA, and LdtB) and two inflammatory cytokines (IL-6 and TNF- α), with rifampicin and brazilin used as reference ligands.

Results: Mitragynine showed favorable drug-likeness, demonstrating transport and pharmacokinetic properties, high gastrointestinal absorption, and blood-brain barrier permeability. Molecular docking revealed moderate binding affinity to the Mtb targets isocitrate lyase and BioA, though weaker than rifampicin. Notably, mitragynine exhibited stronger binding to IL-6 and TNF- α than brazilin, suggesting a greater immunomodulatory potential.

Conclusion: These results position mitragynine as a promising dual-function compound with potential adjunctive therapeutic value for Mtb and inflammation-related pathology. Further studies are warranted to validate these computational findings.

Keywords: Mitragynine, Tuberculosis, Immunomodulation, Molecular docking, Natural compounds

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Tuberculosis (TB) remains a significant global health challenge and is the leading cause of death from infectious diseases worldwide. In 2023, there were an estimated 936,000 new TB cases and nearly 86,000 deaths, bringing the total to about 10.8 million, up from 10.7 million in 2022 and 10.1 million in 2020 [1]. This problem is

exacerbated by the resistance of *Mycobacterium tuberculosis* (Mtb) to various types of drugs including first-line medications such as isoniazid (INH), rifampicin (RIF), ethambutol, and pyrazinamide, as well as second-line drugs such as fluoroquinolones and injectables like capreomycin, kanamycin, and amikacin; leading to multidrug-resistant TB (MDR-TB), which has reached half a million cases worldwide [2]. The

complex interactions among immune cell populations in TB pathogenesis highlight the need to identify new compounds that can prevent or reduce the risk of bacterial resistance and hyperinflammation in patients.

Kratom (*Mitragyna speciosa*) is a tropical evergreen tree from the *Rubiaceae* family, native to Southeast Asia, and traditionally used for pain relief, mood enhancement, and the management of opioid addiction, particularly through its leaves. The main bioactive compounds in kratom leaves are mitragynine, 7-hydroxymitragynine, polyphenols, and flavonoids. Other bioactive compounds include *speciociliatine*, *speciogynine*, *mitraciliatine*, *paynantheine*, *isopaynantheine*, and *epiallo-isopaynantheine*. Mitragynine is the most abundant alkaloid in kratom, and along with its derivatives, it exhibits analgesic effects through interactions with opioid receptors and α -adrenoceptors. These compounds show potential for pain management with reduced side effects compared to classical opioids. In addition, mitragynine also plays a role in the adrenergic and serotonergic systems [3].

Beyond analgesia, kratom extracts have demonstrated significant antimicrobial and immunomodulatory properties. *In vitro* studies indicate that methanol extracts inhibit the growth of pathogens, including *Escherichia coli* and *Streptococcus pneumoniae*. At the same time, *in silico* analyses suggest that quercetin, a secondary metabolite, may mediate antibacterial effects through interaction with the ATP1 protein [4]. *In vivo* studies in tilapia further reveal enhanced immunity, antioxidant capacity, and disease resistance [5]. Anti-inflammatory effects have been validated in rodent models, in which kratom extracts reduced carrageenan-induced edema [6] and suppressed inflammatory mediators such as COX-2, PGE2 [7], and nitric oxide [8].

Despite these antibacterial and anti-inflammatory activities, the potential of mitragynine as an anti-tuberculosis agent remains underexplored. Tuberculosis presents a dual challenge of bacterial persistence and pathological inflammation, making mitragynine a compelling candidate for adjunctive TB therapy. Furthermore, as a native plant in high-TB-burden regions such as Indonesia, kratom offers a locally relevant and sustainable resource for developing accessible treatments, thereby reducing dependency on imported pharmaceuticals.

Tuberculosis remains a global health threat, with high rates of infection and drug resistance,

creating a pressing local need for new therapeutic strategies. Investigating the potential of mitragynine from kratom leaf extract as an adjunctive anti-TB agent offers a locally relevant, resource-based approach.

This study employs an *in silico* molecular docking approach to investigate mitragynine's binding interactions with key Mtb-related proteins, Isocitrate lyase, RmlD, BioA, and LdtB, and the pro-inflammatory cytokines IL-6 and TNF- α . Supported by pharmacokinetic absorption, distribution, metabolism, and excretion (ADME) analysis, this computational investigation aims to elucidate mitragynine's dual anti-TB and immunomodulatory potential, providing a foundation for future experimental validation and therapeutic development.

METHODS

Absorption, distribution, metabolism and excretion (ADME) test

The absorption, distribution, metabolism, and excretion (ADME) tests were conducted to determine the best alkaloidal content of *M. speciosa*. In this study, ADME tests were performed on the most abundant alkaloids in kratom leaf extract, namely mitragynine and 7-hydroxymitragynine, using the SwissADME server. The parameters analyzed were adopted from previous studies. They included molecular weight (g/mol), topological polar surface area (TPSA), ESOL (estimated solubility) class, Silicos-IT class, absorption score (GI), blood–brain barrier (BBB) permeability, Lipinski score, bioavailability score, and synthetic accessibility [9].

Molecular docking simulation

Molecular docking is a crucial computational method in drug discovery, used to predict protein–ligand interactions and binding affinities. It enables virtual high-throughput screening of large compound libraries to identify potential drug candidates [10]. This study used a molecular docking–based computational approach to evaluate the potential of mitragynine as an antibacterial and immunomodulatory agent by comparing it to native ligands, namely rifampicin for antibacterial activity and brazilin for immunomodulatory effects. Protein targets used for antibacterial analysis were isocitrate lyase (PDB ID: 1XG4), RmlD (PDB ID: 1VL0), BioA (PDB ID: 3TFU), and LdtB (PDB ID: 3TUR). Isocitrate lyase was chosen because it plays a key role in the glyoxylate bypass pathway, which is essential for bacterial survival under

environmental stress [11]. RmlD (dTDP-6-deoxy-L-lyxo-4-hexulose reductase) and BioA (adenosylmethionine-8-amino-7-oxononanoate aminotransferase), which are involved in carbohydrate and biotin biosynthesis, respectively, are potential targets for disrupting bacterial metabolic pathways [12], while LdtB (L, D-transpeptidase LdtB) plays a role in cell wall biogenesis and peptidoglycan layer biosynthesis pathways [13]. Rifampicin was used as a control in docking simulations against these targets to compare mitragynine's binding potential.

For immunomodulatory studies, IL-6 (PDB ID: 1ALU) and TNF- α (PDB ID: 2AZ5) were used as targets. Interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) are major pro-inflammatory cytokines that play key roles in immune and inflammatory responses. Brazilin was used as a comparative native ligand to mitragynine, given brazilin's established role in modulating immune responses [14]. The molecular docking process was conducted using AutoDock Vina, chosen for its efficiency, accuracy, and user-friendly energy-based scoring system for predicting binding poses and ligand affinity [9]. Before simulation, all target proteins were prepared by removing water molecules and native ligands and by adding polar hydrogen atoms to facilitate hydrogen bonding. The ligands mitragynine, rifampicin, and brazilin were structurally optimized to their minimum-energy conformations using ChemDraw software with the MM2 force field to ensure stable docking inputs. Docking simulations were performed with AutoDock and further validated using the

HDOCK Server over a 100 ns repetition, with receptors prepared by eliminating non-essential water molecules and ligands. Results were analyzed based on binding energy (kcal/mol) and critical residue interactions, where lower energy values indicated stronger affinity and greater therapeutic potential [15]. The HDOCK server (<http://hdock.phys.hust.edu.cn/>) provided visualization support, with confidence scores interpreted as follows: > 0.7 (high binding probability), 0.5 – 0.7 (possible binding), and < 0.5 (unlikely binding) [16]. Interaction profiling, including hydrogen bond analysis and bond distances (in Ångström units), was performed using Biovia Discovery Studio and the Protein–Ligand Interaction Profiler Server to compare interactions between mitragynine and the target receptors; the results are shown in Table 1.

RESULTS

ADME test

Based on the ADME analysis, the pharmacokinetic parameters of the two main compounds, mitragynine and 7-hydroxymitragynine, demonstrated bioavailability and solubility profiles relevant to drug development. The ADME test profiles for mitragynine and 7-hydroxymitragynine can be seen in Table 2, while the complete output from the SwissADME server is shown in Figure 1 (mitragynine) and Figure 2 (7-hydroxymitragynine).

Table 1: The ligands and receptors involved in the molecular docking simulations

Ligand	Pubchem ID	Target receptors	PDB ID
Mitragynine	3034396	Isocitrate lyase	1XG4
Rifampicin	135398735	RmlD	1VL0
Brazilin	73384	BioA	3TFU
		LdtB	3TUR
		IL-6	1ALU
		TNF- α	2AZ5

Table 2: ADME test profiles of mitragynine and 7-hydroxymitragynine

Parameter	Mitragynine	7-hydroxymitragynine
PubChem ID	3034396	44301524
Molecular weight (g/mol)	398.5	414.5
TPSA (Å ²)	63.79	80.59
ESOL class	-4.29	-3.59
Silicos-IT class	Moderate solubility	Moderate solubility
Absorption score (GI)	High	High
BBB permeability	Yes	No
Lipinski score	0	0
Bioavailability score	0.55	0.55
Synthetic accessibility	4.99	5.21

TPSA: Topological Polar Surface Area; ESOL: Estimated Solubility; BBB (Blood-Brain Barrier)

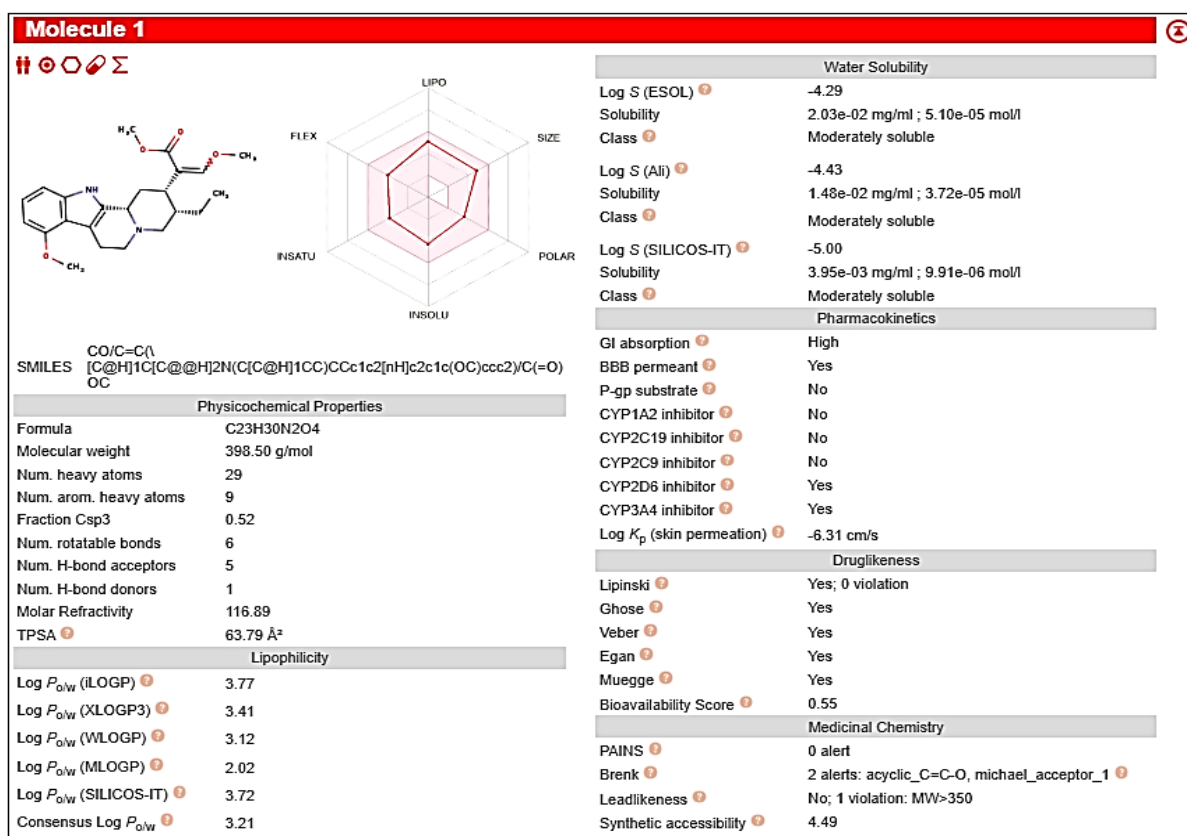


Figure 1: ADME test results for mitragynine using the Swiss ADME server

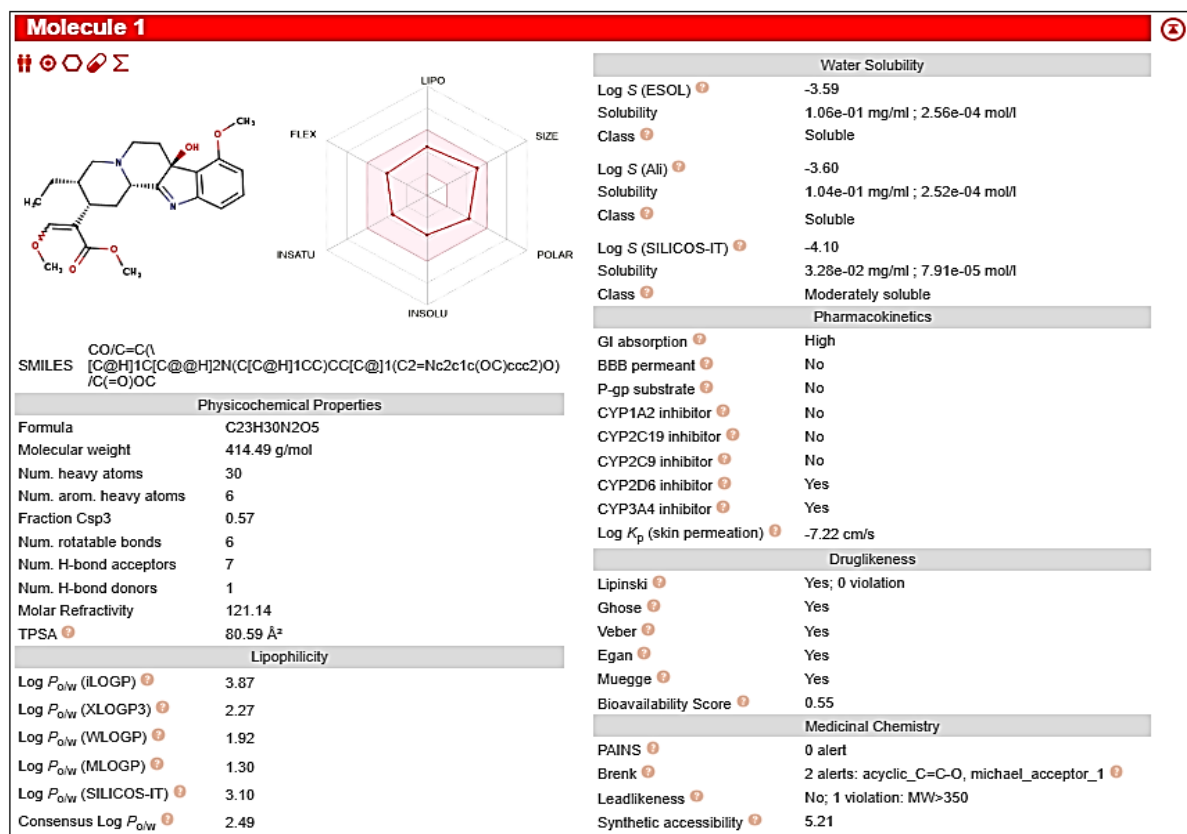


Figure 2: ADME test results for 7-hydroxymitragynine using the Swiss ADME server

```

-----MM2 Minimization-----
Pi System: 21 14 12 6 18 19 24 25 26
Pi System: 4 22 17 23
Warning: Some parameters are guessed (Quality = 1).
Iteration 785: Minimization terminated normally because the gradient norm is less than the minimum gradient norm
Stretch: 3.1179
Bend: 20.8957
Stretch-Bend: 0.5116
Torsion: 2.2521
Non-1,4 VDW: -5.1058
1,4 VDW: 27.1482
Dipole/Dipole: 1.0273
Total Energy: 49.8469 kcal/mol
Calculation completed
-----

-----MM2 Minimization-----
Pi System: 47 45 6 44 8 38 39 37 43 40 46 50 16 49 57
Pi System: 30 33 41 12 48 56
Warning: Some parameters are guessed (Quality = 1).
Iteration 2843: Minimization terminated normally because the gradient norm is less than the minimum gradient norm
Stretch: 8.6500
Bend: 37.4695
Stretch-Bend: 1.7584
Torsion: 18.7547
Non-1,4 VDW: -17.3356
1,4 VDW: 45.6011
Dipole/Dipole: -3.6905
Total Energy: 91.2176 kcal/mol
Calculation completed
-----

-----MM2 Minimization-----
Pi System: 16 12 13 19 20 21
Pi System: 14 9 10 15 17 18
Warning: Some parameters are guessed (Quality = 1).
Iteration 319: Minimization terminated normally because the gradient norm is less than the minimum gradient norm
Stretch: 1.1992
Bend: 10.7906
Stretch-Bend: -0.1762
Torsion: -10.7683
Non-1,4 VDW: -9.9719
1,4 VDW: 12.6622
Dipole/Dipole: 1.5925
Total Energy: 5.3282 kcal/mol
Calculation completed
-----

```

Figure 3: Energy minimization of mitragynine, rifampicin, and brasilin with ChemDraw application

Mitragynine has a molecular weight of 398.5 g/mol and a Topological Polar Surface Area (TPSA) of 63.79 Å², while 7-hydroxymitragynine has a higher molecular weight of 414.5 g/mol and a larger TPSA of 80.59 Å². The Estimated Solubility (ESOL) log S value was -4.29 for mitragynine and -3.59 for 7-hydroxymitragynine. According to the Silicos-IT class, both compounds were categorized as having moderate solubility. Based on the gastrointestinal (GI) absorption score, both were categorized as high. A difference in BBB permeability was observed: mitragynine showed permeability, whereas 7-hydroxymitragynine did not. The Lipinski score was zero for both compounds, and the bioavailability score was 0.55. For synthetic accessibility, mitragynine had a score of 4.99, slightly lower than that of 7-hydroxymitragynine (5.21).

Molecular docking simulation: ligand preparation

Energy minimization was performed on the three ligands before the molecular docking simulation.

The total energy of mitragynine was 49.84 kcal/mol. The total energy of rifampicin reached 91.22 kcal/mol after minimization. Minimization of the brasilin molecule showed a total energy of 5.33 kcal/mol. The minimization process concluded when the gradient norm fell below the specified minimum threshold, indicating convergence and stable configurations for all ligands. Figure 3 shows the energy-minimized structures of mitragynine, rifampicin, and brasilin generated in ChemDraw.

Docking of mitragynine on Mtb-related molecules

The interaction values (binding affinities) of mitragynine with the four Mtb receptors were lower than those of rifampicin (Table 3). The Root Mean Square Deviation (RMSD) values from docking mitragynine with isocitrate lyase (46.37 Å) and LdtB (28.8 Å) were lower than the value obtained for rifampicin with the same receptors (55.97 Å and 41.46 Å, respectively). For isocitrate lyase (1XG4), the interaction value for mitragynine was -184.84 kcal/mol. Residues

Thr59A, Thr63A, Thr59C, and Asp65D formed hydrogen bonds, while residues Leu64C and Arg72B formed hydrophobic bonds.

For RmlD (1VL0), the interaction value for mitragynine was -202.6 kcal/mol. Residues Phe239A, Val175A, and Leu151A formed hydrogen bonds, whereas residue Val174A formed a hydrophobic bond. For BioA (3TFU), the interaction value for mitragynine was -233.77 kcal/mol. The Gly225B residue formed a hydrogen bond, while residues Trp64B, Ala226B, Phe402B, Met229B, Tyr25B, and Met314A formed hydrophobic bonds. For LdtB (3TUR), the interaction value for mitragynine was -157.91 kcal/mol, and no residues forming hydrogen or hydrophobic bonds were identified. Visualization of the docking simulations of mitragynine and rifampicin with their target receptors is shown in Figure 4. In contrast, Figure 5 shows the docking simulations of mitragynine and brazilin with their target receptors.

Docking of mitragynine as an immunomodulator

The interaction of mitragynine with the immunomodulatory receptors showed a higher (more negative) affinity value than brazilin (Table 3). The RMSD value from docking mitragynine with TNF- α (69.67 Å) was lower than that of brazilin (85.24 Å). For IL-6 (1ALU), the interaction value for mitragynine was -136.21 kcal/mol. Residues Gln156A, Glu42A, Asp160A, Gln159A, Arg104A, and Thr163A formed hydrogen bonds, while the Glu106A residue formed a hydrophobic bond. For TNF- α (2AZ5), the interaction value for mitragynine was -162.54 kcal/mol. Residues Gln61D, Tyr119C, Leu120C, and Tyr151C formed hydrogen bonds. Visualization of the docking simulations of mitragynine and brazilin with their target receptors is shown in Figure 5.

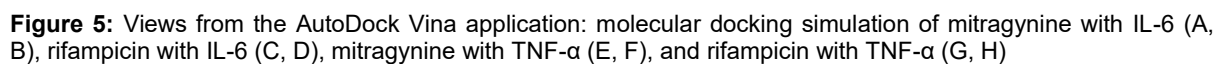
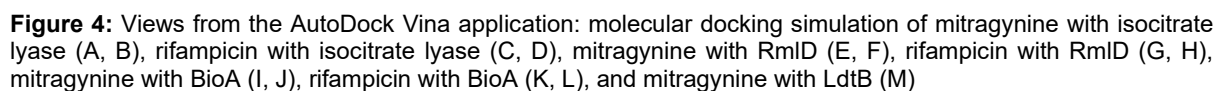
DISCUSSION

The ADME profile analysis indicated good pharmacokinetic properties for both compounds. The TPSA values below 140 Å^2 indicate good permeability across biological membranes and good oral bioavailability. The ESOL values classified both compounds as moderately soluble, which, combined with high GI absorption, suggests potential for systemic bioavailability [17].

The main finding was the BBB permeability of mitragynine, a property that 7-hydroxymitragynine does not possess, even though both are the most abundant alkaloid compounds in kratom leaf extract. This suggests possible central nervous system (CNS) activity that may enhance its immunomodulatory or analgesic effects, consistent with its known interactions with opioid and adrenergic receptors in the brain [18]. Both compounds scored zero in the Lipinski rule, indicating good drug-likeness, and had a bioavailability score of 0.55, suggesting moderate bioavailability potential. Their synthetic accessibility scores were within an acceptable range [9]. These characteristics enhance the viability of mitragynine for medicinal development. The energy minimization process is essential for locating the lowest-energy configuration and serves as a reliable starting point for accurate protein–ligand interaction modeling. The convergence of the gradient norm below the threshold confirms that the molecules have reached a locally minimized energy conformation, indicating optimized molecular geometries in their most stable state, a crucial criterion for reliable docking simulations. This optimization is standard in structure-based drug design and is necessary for predicting binding poses and properties [19].

Table 3: Docking simulation profiles of mitragynine with related receptors

Purpose of analysis	Ligand	Receptor	Interaction value (kcal/mol)	Confidence value	RMSD value (Å)
Antibacterial potential	Mitragynine	Isocitrate lyase	-184.84	0.67	46.37
		RmlD	-202.6	0.74	51.4
		BioA	-233.77	0.84	49.57
		LdtB	-157.91	0.53	28.8
	Rifampicin	Isocitrate lyase	-193.77	0.7	55.97
		RmlD	-262.01	0.9	44.49
		BioA	-290.65	0.94	41.72
		LdtB	-205.8	0.75	41.46
Immunomodulator potential	Mitragynine	IL-6	-136.21	0.43	37.1
		TNF- α	-162.54	0.56	69.67
	Brazilin	IL-6	-130.72	0.4	34.65
		TNF- α	-154.84	0.52	85.24



The stable energy states of all ligands enabled a fair comparison of docking outcomes.

The docking results indicate that mitragynine has a multi-target binding profile against key Mtb enzymes, although with generally weaker affinity than the control drug rifampicin. The moderate binding affinity of mitragynine toward isocitrate lyase (-184.84 kcal/mol), though lower than that of rifampicin, involved interactions with key residues (Thr59A, Thr63A). The lower RMSD value may imply greater stability of the ligand–receptor complex, suggesting that mitragynine may interact with the essential glyoxylate pathway. For RmlD, the lower affinity of mitragynine (-202.6 kcal/mol) compared to rifampicin suggests it is less effective at inhibiting this enzyme. The high RMSD value (51.4 Å) indicates a less stable docking pose. However, the interaction with shared residues such as Trp214A shows some similarity in binding, and its complex mode of action may still confer pharmacological significance through other targets. Regarding BioA, the absence of strong hydrogen bonding, as seen with rifampicin, and the higher RMSD value suggest a weaker modulatory effect. While mitragynine binds to BioA, its reliance on non-covalent interactions and its higher binding energy suggest it is unlikely to be an effective inhibitor in its current state. For LdtB, mitragynine exhibited the weakest binding and no significant interactions, limiting its potential as a direct inhibitor. However, the relatively lower RMSD value suggests conformational compatibility, offering a foundation for chemical modification or combinatory therapy to target cell wall synthesis [9]. Mitragynine demonstrated a stronger binding affinity for the pro-inflammatory cytokines IL-6 and TNF- α than the native ligand, braziliin. For IL-6, the higher affinity (-136.21 kcal/mol) and the involvement of key residues such as Arg104A and Glu106A correlate with its known anti-inflammatory activity via COX-2 and PGE2 inhibition [7]. IL-6 is a significant cytokine in TB immunopathology, and its inhibition has clinical utility in diseases such as rheumatoid arthritis and COVID-19 [20], positioning mitragynine as a promising natural suppressor of hyperinflammation. The strong binding of mitragynine to TNF- α (-162.54 kcal/mol), supported by multiple hydrogen bonds with key residues, further enhances its potential as an immunomodulator. TNF- α is a primary modulator of inflammation and granuloma formation in TB. The fact that mitragynine engages residues critical for cytokine function supports its potential role in modulating destructive inflammatory responses in TB. Given the widespread clinical use of TNF- α inhibitors [21], plant-derived

alternatives such as mitragynine could provide safer, more affordable options. Its dual activity against both IL-6 and TNF- α identifies a broad anti-inflammatory spectrum.

The high RMSD values across most simulations indicate significant conformational differences from the reference structures. Values above 2 Å suggest notable deviation, while values greater than 5 Å signify low stability and substantial conformational change. Factors such as structural flexibility, intermolecular forces, temperature, and pH can influence these values. The high RMSD values necessitate further optimization through techniques such as molecular dynamics simulations to improve interaction efficiency and validate complex stability [9]. This study provides initial *in silico* insights, but experimental validation is required to confirm these findings.

Limitations of the study and future directions

This study is primarily limited by its reliance on static molecular docking as the main analytical framework, which does not account for conformational flexibility, solvent effects, or the dynamic conditions of a living system, thereby restricting the interpretation of binding affinity and specificity. All predicted docking poses showed RMSD values exceeding 2 Å, indicating substantial deviation from true binding conformations and raising concerns about the validity of the results. Because its interactions are not entirely validated, rifampicin may bind to proteins other than the intended target, leading to misleading results. This makes it difficult to attribute observed effects solely to the target protein of interest. The choice to use rifampicin, despite its potential for minor off-target effects at high doses or in specific systems, is a cost–benefit decision based on its potent and selective action at clinically relevant or specific experimental concentrations. To address these limitations, future studies should incorporate more dynamic approaches, such as molecular dynamics simulations for conformational assessment; employ well-characterized natural ligands or structurally related inactive analogs as controls; and refine docking accuracy through flexible receptor modeling, improved scoring functions, and pose refinement. Despite these constraints, the strong binding affinity observed for key pro-inflammatory cytokines such as TNF- α and IL-6 suggests promising immunomodulatory potential, providing a preliminary yet meaningful foundation for subsequent *in vitro* and *in vivo* validation and future translational research.

CONCLUSION

This study provides preliminary evidence that mitragynine, a natural alkaloid of *Mitragyna speciosa*, may serve as a dual-action compound with immunomodulatory and anti-tuberculosis activity. In silico docking showed favorable ADME properties and strong binding affinities to *Mycobacterium tuberculosis* enzymes (isocitrate lyase and BioA) and cytokines (IL-6, TNF- α). Although less potent than rifampicin or brazilin, mitragynine's multi-target profile, blood–brain barrier permeability, and natural origin make it worthy of further exploration. Notably, rifampicin's protein interactions outside its primary bacterial target are not entirely validated, which raises the risk of misleading results. Future studies should adopt more appropriate control strategies, such as natural ligands, inactive analogs, or established positive controls, to ensure reliable benchmarking in evaluating mitragynine's activity. It is now recommended that rifamycin derivatives, such as rifabutin or rifapentine, or mechanistically distinct drugs, such as isoniazid (a cell wall synthesis inhibitor), to validate target specificity. Researchers can also use non-endogenous RNA polymerase inhibitors, such as myxopyrronine or lipiarmycin, which bind at a different site than rifampin. All docking results in this study showed RMSD values above 2 Å, indicating that the predicted binding poses may not accurately represent biological interactions. This limitation reflects the constraints of static docking and underscores the need for refinement through flexible receptor models, improved scoring functions, or molecular dynamics simulations. Such strategies can improve docking accuracy and enhance predictive value. Despite these constraints, the findings highlight mitragynine's potential as a natural compound–derived adjunct treatment for tuberculosis and immunological conditions, pending confirmation through *in vitro* and *in vivo* validation.

DECLARATIONS

Acknowledgement/Funding

This research was funded by the Director General of Health Workers, Ministry of Health of the Republic of Indonesia, grant number HL.02.03/F/2582/2024.

Use of Artificial Intelligence

The authors utilized artificial intelligence (AI) technology (ChatGPT, Deepsek, and Quilbot) exclusively for linguistic refinement and

proofreading of the manuscript. The core intellectual ideas, research data, analysis, and scholarly interpretations are entirely original to the authors. The authors have thoroughly reviewed and endorsed the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ari Nuswantoro made a substantial contribution to data collection and the concept or design of the article. Jusak Nugraha made a significant contribution to supervising the article writing. Sri Agus Sudjarwo revised the article critically for important intellectual content. Purwo Sri Rejeki approved the version for publication. Dinasti Aprillia helped collect data for the article. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2024 [Internet]. 2024. Available from: <https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2024>.
2. Iacobino A, Fattorini L, Giannoni F. Drug-resistant tuberculosis 2020: where we stand. *Applied Sci* 2020; 10(6): 2153. <https://doi.org/10.3390/app10062153>.
3. Permatasari HK, Nugrahenny D, Lestari B, Hakim RW, Zulkarnain Z, Anshory M, Nurkolis F. Kratom (*Mitragyna speciosa*) in cancer chemoprevention – A provocative functional food perspective. *J Agric Food Res* 2025; 24: 102525. DOI. 10.1016/J.JAFR.2025.102525.
4. Salim HM, Puspitarini MD, Setiwati Y, Shimabukuro M. Antibacterial mechanism of Kratom (*Mitragyna speciosa*) methanol extract on *Streptococcus pneumoniae* and *Eschericia coli* bacteria. *Biomolecular Health Sci J* 2021; 4(2): 99–103. Doi. 10.20473/BHSJ.V4I2.28933.
5. Paankhao N, Sangsawang A, Kantha P, Paankhao S, Promsee K, Soontara C, Kongsriprapan S, Srisapoom P, Kumwan B, Meachasompop P, et al. Antioxidant and antibacterial efficiency of the ethanolic leaf extract of *Trop J Pharm Res*, February 2026; 25(2): 169

- Kratom (*Mitragyna speciosa* (Korth.) Havil) and its effects on growth, health, and disease resistance against *Edwardsiella tarda* infection in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol* 2024; 152: 109771. DOI. 10.1016/j.fsi.2024.109771.
6. Salim HM, Choirotussanijjah, Awwalia ES, Alam IP. Anti-inflammatory effects and potential mechanisms of *Mitragyna speciosa* methanol extract on λ -karagenan-induced inflammation model. *Bali Medical J* 2022; 11(3): 1172–1175. DOI. 10.15562/BMJ.V11I3.3535.
 7. Utar Z, Majid MIA, Adenan MI, Jamil MFA, Lan TM. Mitragynine inhibits the COX-2 mRNA expression and prostaglandin E_2 production induced by lipopolysaccharide in RAW264.7 macrophage cells. *J Ethnopharmacol* 2011; 136(1): 75–82. Doi. 10.1016/J.JEP.2011.04.011.
 8. Tohar N, Shilpi JA, Sivasothy Y, Ahmad S, Awang K. Chemical constituents and nitric oxide inhibitory activity of supercritical carbon dioxide extracts from *Mitragyna speciosa* leaves. *Arab J Chem* 2019; 12(3): 350–359. Doi. 10.1016/J.ARABJC.2016.09.005.
 9. Fan C, Basharat Z, Mah K, Wei CR. Computational approach for drug discovery against *Gardnerella vaginalis* in quest for safer and effective treatments for bacterial vaginosis. *Scientific Reports* 2024; 14(1): 1–14. Doi. 10.1038/s41598-024-68443-2.
 10. Onoja JO, Unamba JC, Eleje CC, Tjegbe MJM. Inhibitory effect of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burt on prostaglandin/leukotrienes synthesis, and in silico analysis of HPLC-identified compounds. *Trop J Pharm Res* 2025; 24(5): 719–730. Doi. 10.4314/TJPR.V24I5.10.
 11. Lee YV, Choi SB, Wahab HA, Lim TS, Choong YS. Applications of ensemble docking in potential inhibitor screening for *Mycobacterium tuberculosis* isocitrate lyase using a local plant database. *J Chem Inf Model* 2019; 59(5): 2487–2495. Doi. 10.1021/ACS.JCIM.8B00963.
 12. Liu F, Dawadi S, Maize KM, Dai R, Park SW, Schnappinger D, Finzel B, Aldrich C. Structure-based optimization of pyridoxal 5'-phosphate-dependent transaminase enzyme (BioA) inhibitors that target biotin biosynthesis in *Mycobacterium tuberculosis*. *J Med Chem* 2017; 60(13): 5507–5520. Doi. 10.1021/ACS.JMEDCHEM.7B00189.
 13. Schoonmaker MK, Bishai WR, Lamichhanea G. Nonclassical transpeptidases of *Mycobacterium tuberculosis* alter cell size, morphology, the cytosolic matrix, protein localization, virulence, and resistance to β -lactams. *J Bacteriol* 2014; 196(7): 1394–1402. Doi. 10.1128/JB.01396-13.
 14. Nava-Tapia DA, Cayetano-Salazar L, Herrera-Zúñiga LD, Bello-Martínez J, Mendoza-Catalán MA, Navarro-Tito N. Brazilin: Biological activities and therapeutic potential in chronic degenerative diseases and cancer. *Pharmacol Res* 2022; 175: 106023. Doi. 10.1016/J.PHRS.2021.106023.
 15. Guterres H, Im W. Improving protein-ligand docking results with high-throughput molecular dynamics simulations. *J Chem Inf Model* 2020; 60(4): 2189–2198. Doi. 10.1021/ACS.JCIM.0C00057.
 16. Li H, Huang E, Zhang Y, Huang SY, Xiao Y. HDock update for modeling protein-RNA/DNA complex structures. *Protein Sci* 2022; 31(11): e4441. Doi. 10.1002/PRO.4441.
 17. Rai M, Singh AV, Paudel N, Kanase A, Falletta E, Kerkar P, Heyda J, Barghash R, Pratap Singh S, Soos M. Herbal concoction unveiled: A computational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMS). *Curr Res Toxicol* 2023; 5: 100118. Doi. 10.1016/J.CRTOX.2023.100118.
 18. Dohárszky A, Vági E, Könczöl Á, Simon A, Várnagy E, Muratov M, Steiger K, Várnai B, Béni S, Riethmüller E, et al. Kratom alkaloids: A blood–brain barrier specific membrane permeability assay-guided isolation and cyclodextrin complexation study. *Molecules* 2024; 29(22): 5302. Doi. 10.3390/MOLECULES29225302.
 19. Agóni C, Fernández-Díaz R, Timmons PB, Adelfio A, Gómez H, Shields DC. Molecular modelling in bioactive peptide discovery and characterisation. *Biomolecules* 2025; 15(4): 524. Doi. 10.3390/BIOM15040524.
 20. Du P, Geng J, Wang F, Chen X, Huang Z, Wang Y. Role of IL-6 inhibitor in treatment of COVID-19-related cytokine release syndrome. *Int J Med Sci* 2021; 18(6): 1356. doi. 10.7150/IJMS.53564.
 21. Yuk JM, Kim JK, Kim IS, Jo EK. TNF in human tuberculosis: a double-edged sword. *Immune Netw* 2024; 24(1): e4. Doi. 10.4110/IN.2024.24.E4.