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In Silico Investigation of Plant-Derived Anti-Tuberculosis Compounds Targeting Kat G Protein: Molecular Docking and Dynamics Approaches Compared with Isoniazid

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Abstract

Tuberculosis (TB) is a global infectious disease caused by *Mycobacterium tuberculosis,* causing millions of deaths annually. Tuberculosis has become severe public health threat. The conventional treatment of tuberculosis often results that first line of therapeutic drug has unwanted complications and face significant health challenges in the emergence of resistance to multiple drugs, Kat G is the crucial pathogenicity determinant which it increases bacterium ability to establish infection of the strain *Mycobacterium tuberculosis*. Therefore, in this study designed to examine the inhibitory effects of bioactive active compound on Kat G enzyme as determined through in silico studies. Molecular properties, molecular docking, molecular dynamic stimulation were performed to evaluate new anti-tuberculosis therapeutics. Plant- derived bioactive compounds such as Oleanolic acid, Ursolic acid, Baicalein, Triterpenoid and Thymol have binding affinity which has potential anti-tuberculosis activity of the compound compared to control drug. The finding indicates that Oleanolic acid had a most potent anti- tuberculosis activity by inhibiting Kat G compared to other compounds with the most optimal binding affinity and molecular dynamic stimulation interaction properties. Thus, we recommend that Oleanolic acid from L. aspera, has a potent anti-tuberculosis drug offering new insights into drug resistance against Kat G inhibition of *Mycobacterium tuberculosis.*

Key words : *In silico, Mycobacterium tuberculosis*, Kat G, Bioactive compound, Drug resistance.

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Tuberculosis (TB) is an infectious disease which causes called Mycobacterium tuberculosis and it persists as a prominent public health concern worldwide. TB is amenable to prevention and successfully treated diseases[13]. In the year of 2023 there was approximately 10.8 million new cases and 1.25 million deaths, so this is known as one of the top infectious killer diseases, representing considerable burden on population health[7]. And about 70 % of death rate occurs with the Detrimental outcomes associated with (Human Immunodeficiency virus) HIV-TB[19]. Despite substantial development in treatment and diagnosis, majority 66% of cases contribute by Bangladesh (4%), China (9%), Indonesia (8%), India (27%), Philippines (6%), Pakistan (5%), Nigeria (4%) and South Africa (3%) (World Health Organization, 2023)[12]. *Mycobacterium tuberculosis* causes tuberculosis (TB), is mostly spread through the air when an infected person coughs, sneezes, talks, or vocalizes. This disease is released into the atmosphere in the form of tiny droplets, which nearby people may then inhale and get infected. The transmission cascade may be disrupted by interventions that target any one of these three processes; on the other hand, variables (or "catalysts") that promote these events may make the spread of TB worse. Factors affecting Mycobacterium tuberculosis transmission rates, contagiousness, and susceptibility and it affects nearly every organ in human body[5]. However, we still lack answers to many important questions regarding the pathogenesis and any immunological correlates of disease prevention, such as those raised by E.L. Trudeau over a century ago[4]. Due to its direct influence on TB diagnosis and treatment, the number of TB cases has been sharply rising since the advent of COVID-19. The World Health Organization (WHO) has rescheduled the 2025 target of TB eradication to 2035 in light of the present circumstances[6]. Millions of people die from tuberculosis every year, despite tremendous progress in our understanding of the disease. The bacteria can withstand immune reactions and thrive inside host macrophages because to its special cell wall, which is rich in mycolic acids. Both active and latent tuberculosis infections are facilitated by the host immune resistance.

The early diagnosis of TB is sputum smear microscopy and by the molecular diagnosis Line probe assay (LPA's) and PCR based assay and X-Ray by radiological method[21]. And it shows the symptoms like Chronic cough, Hemoptysis, Fever, Weight loss, regional lymphadenopathy, Thoracoabdominal pain. A Treatment is initiated upon positive identification and confirmation of the pathogen TB. The therapeutic protocols are Similar approaches that are applied to the both pulmonary and extrapulmonary infection[24].

At the early stage of tuberculosis infections, drug Rifampicin (RIF), Isoniazid (INH), drugs are given for first two months. Ethambutol (EMB), Pyrazinamide (PZA), Rifampicin (RIF) and Isoniazid (INH) used subsequently next 4 months at the second stage of tuberculosis infections[3]. By using first line of regimens of Tuberculosis (Tb) commonly causes diverse side effects like Hepatocellular damage (2.4%), Ototoxicity (1.7%), Functional liver disturbance (4.9%), Hyperuricemia

(2.6%), Psychiatric changes (0.7%) and Cutaneous infection (0.6%)[8]. So, the advancement of new anti-TB novel drug is needed.

Phytotherapeutic plants are widely used as not only for culinary applications, but also used to treat multiple health condition and illness. This includes various sources like Ayurveda, Siddha, Unani, these system of medicine has contributed to the discovery of essential drugs that still in use today. Ethnomedicinal plant include variety of plant types and they act as a valuable source of ingredient for the pharmaceutical development. *Leucas aspera* (L. *aspera*) is a common invasive plant which is mostly seen in Tropical Asia and Africa. *L. aspera* belongs to the family *Lamiaceae,* which has about 80 species[23]. This plant contains specific and characteristic bioactive constituent that are thought to have health – enhancing properties and address multiple medical conditions. *L. aspera* has been traditionally used for cold, cough, skin rashes, sore throat, painful swelling, jaundice and asthma. Broad study has proven that this plant has a pharmacological activity such as anti-fungal, anti-oxidant, anti-cancer, anti-venom, hepatoprotective, anti- inflammatory, anti- nociceptive, antiulcer, anti-diabetic, anti-malarial, anti-arthritis and cytotoxic activity[17,20]. Extraction of multiple compounds from various parts of *L. aspera* has been documented including a nicotinic acid, oleanolic acid, ursolic acid, linolenic acid, aliphatic ketones, aliphatic compounds, sterol, flavonoids, triterpenes, phenols were also identified[14]. Accordingly, in the present study is to be find out the bioactive compounds from the natural medicinal plant for their activity as anti-tuberculosis drug candidates. Kat G is a crucial pathogenicity determinant which it increases bacterium ability to establish infection of the strain Mycobacterium tuberculosis. Kat G contributes to the bacterial defense against oxidative stress generated by macrophage mediated immune response[16]. The potent toxic effects of anti-tuberculosis drug is isoniazid is believed to mediate enzyme catalase-peroxidase containing heme specified by Kat G gene of *Mycobacterium tuberculosis*[9]. Gene expression of Kat G in *Mycobacterium tuberculosis,* mycobacterium smegmatis, and Escherichia coli was confirmed through the western blotting analysis[10]. Kat G plays important role in pathogenesis and bacterium survival in the human host.

Selection of chemical properties and Secondary metabolites in L. aspera :

Molecular docking is a computer-aided approach utilized to determine interaction between small molecules and large biomolecules such as DNA, RNA, protein. enzymes and receptor[2]. Identification of secondary metabolites from plant were entirely based on literature survey. Multiple major secondary metabolites from the plant *L. aspera* were utilized in the present study. The SMILE information of secondary metabolites was retrieved from the PubChem database which encompassing Oleanolic acid (CID. 10494), Ursolic acid (CID. 64945), Baicalein (CID. 5281605), Triterpenoid (CID. 451674) and Thymol (CID. 6989). The molecular information of these bioactive compounds was retrieved from IMPPAT webserver.

Ligand structure preparation :

The molecular structure of the secondary metabolites was retrieved from the PubChem database which incorporated oleanolic acid, ursolic acid, baicalein, triterpenoid, thymol and in addition, Isoniazid (CID. 3767) which served and functioned as standard drug for Kat G inhibition. Finally, all molecular structure of selected secondary metabolites and standard drug were exported in SDF (Structure Data File) format for further analysis of molecular docking.

Receptor Protein preparation and optimization :

The selecting approach of receptor protein were entirely dependent on literature survey. Various categories of receptor mycobacterium protein were included in analysis that had 3D structure and various function, particularly 3D structure of Kat G, the target protein sequence are retrieved from RSCB Protein Database Bank with the PDB ID (1SJ2) and the data saved in PDB format. Extraneous compounds like aqueous molecules, non- carbon atoms were eliminated from the Kat G protein structure to derive more appropriate for further molecular docking along with this the protein molecule saved in a PDBQT format[11].

Molecular Docking Analysis and Structural Visualization :

The target protein Kat G was carried out using software PyMOL. The molecular docking was executed using Auto Dock 4.2.6 software. The grid box was configured to the total surface region of Kat G with the spatial parameters X= 72.262, Y= 29.532 and Z= 84.521 with number of modes are 10 and energy range is 4. Analytical evaluation and structural visualization of molecular docking result was done using the software AutoDock Vina.

Molecular Dynamic Stimulation studies :

Molecular docking, molecular dynamic stimulation is carried out to verify stability of protein-ligand complex. Additionally computational approach was employed to estimate key mechanism at molecular level. Further molecular dynamic stimulation was performed using (GROMACS) software. Extended range coulombic interaction was computed by Particle Mesh Ewald (PME) technique[18]. Several key parameters were measured including H-Bond, RMSD, RMSF, SASA, and ROG.

Secondary Metabolites of L aspera :

The secondary metabolites used in this study were its originated from *L. aspera* medicinal plant. A wide range of secondary metabolites has a shared characteristics conjugated ring system taken from PubChem website (Table-1). Further the molecular docking process, the physiochemical properties of secondary metabolites has investigated that includes formula, molecular weight, rotatable bond, H-bond acceptors, H- bond donors and molar refractivity through PubChem website (Table-2). and with the same physiochemical properties also investigated in drug isoniazid (Table-3).

Table-1. Chemical structure of bioactive compounds.

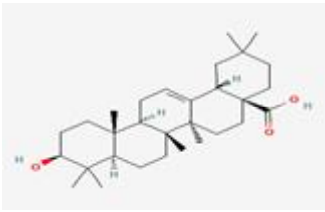
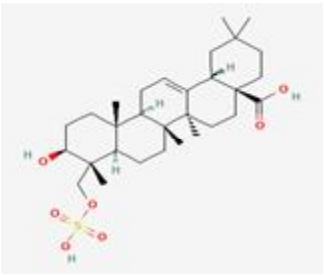
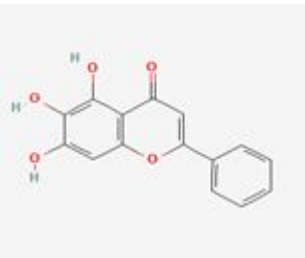
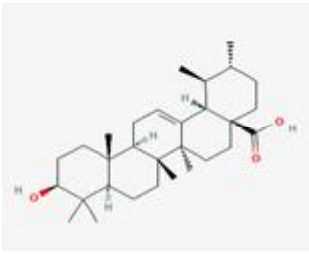
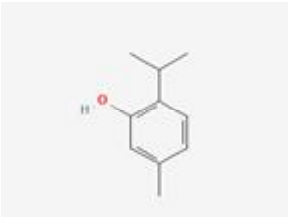
S. No	Name of the compound	Structure	PubChem CID
1	Oleanolic acid		(CID. 10494)
2	Ursolic acid		(CID. 64945)
3	Baicalein		(CID. 5281605)
4	Triterpenoid		(CID. 451674)
5	Thymol		(CID. 6989)

Table-2. Physiochemical properties of bioactive compounds

S. No	Compounds	Formula	Molecular weight	Num. H bond donors	Num. bond acceptors	Num. rotatable bond	Molar Refrac-tivity
1	Oleanolic acid	C30H48O3	456.7 g/mol	2	3	1	111.0
2	Ursolic acid	C30H48O3	456.7 g/mol	2	3	1	111.0
3	Baicalein	C15H10O5	270.24 g/mol	3	5	1	71.2
4	Triterpenoid	C30H48O7S	552.8 g/mol	3	7	4	122.2
5	Thymol	C10H14O	150.22 g/mol	1	1	1	46.15

Table-3. Physiochemical properties of drug Isoniazid.

S. No	Drug	Formula	Molecular weight	Num. H bond donors	Num. bond accep-tors	Num. rotata-ble bond	Molar Refrac-tivity
1	Isoniazid	C6H7N3O	137.14 g/mol	2	3	1	34.73

Molecular Docking :

In molecular docking estimation and binding energy was performed using AutoDock and visualization using AutoDock vina and top-scoring poses were been calculated for each compound and (Table-4) summarizes the top score obtained for each bioactive compound.

Table-4. AutoDock Vina scores on bioactive compounds.

S. No	Compounds	Auto Dock Vina score (kcal/mol)
1	Oleanolic acid	-9.4
2	Ursolic acid	-8.9
3	Baicalein	-8.8
4	Triterpenoid	-8.7
5	Thymol	-7.5

After evaluating drug-likeness properties of bioactive compound, the top binding affinity oleanolic acid and Isoniazid drug is taken for molecular docking using AutoDock vina against Kat G protein of *Mycobacterium tuberculosis*. Based on molecular docking, this bioactive compound in this study exhibited competitive inhibition. It shows bioactive active compound bind to a distinct site of Kat G, which the 3D visualization showed in (Figure 1) and control drug bind to a distinct site of Kat G, which the 3D visualization showed in (Figure 2).

Along with binding interactions, Van der waals and H- bonding interaction is formed between protein – ligand. The 2D structure and interaction visualization of protein-ligand

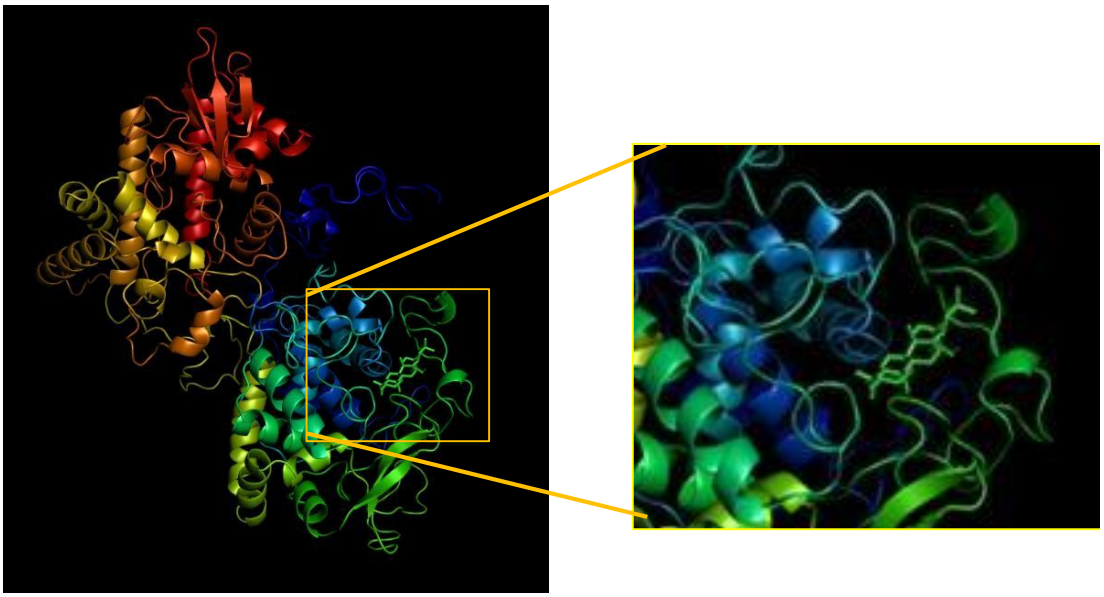


Figure 1. Bioactive compound Oleanolic acid interaction with Kat G

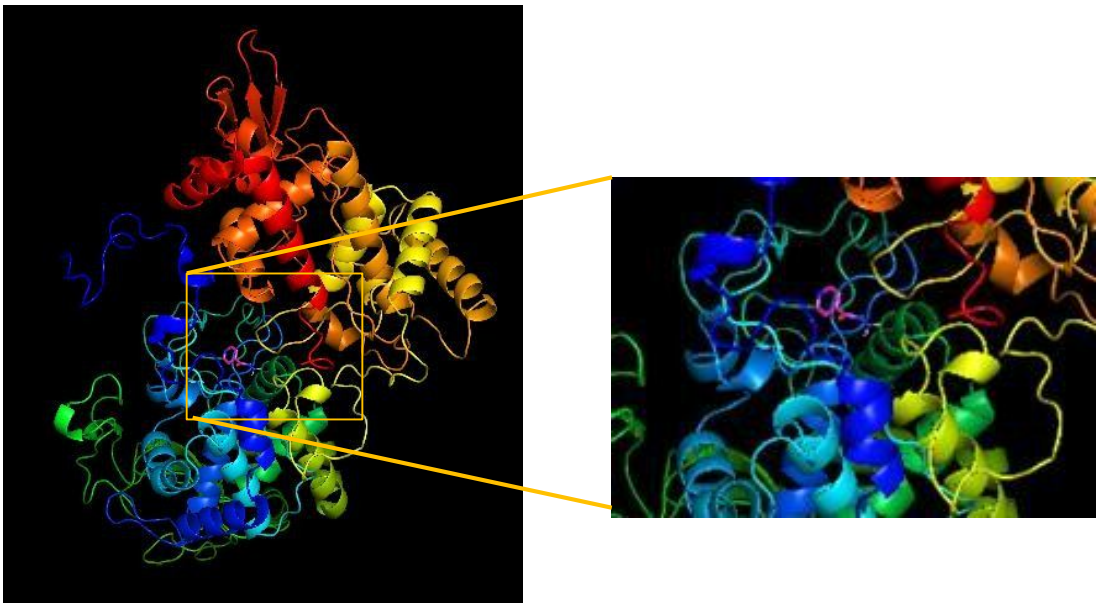


Figure 2. Isoniazid drug interaction with Kat G

of oleanolic acid (Figure 3) and Isoniazid (Figure 4) which both are bound to Kat G individually. This observation demonstrates that the bioactive compound that may influence changes in the bioactivity of Kat G.

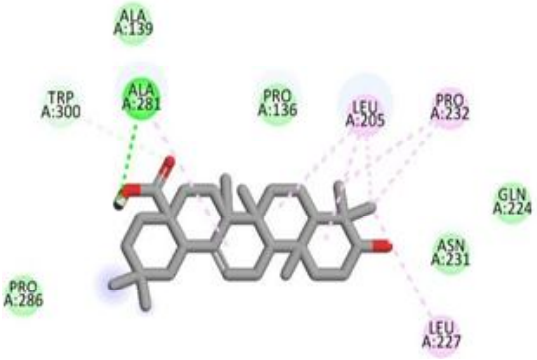


Figure 3. The 2D structure and interaction visualization of protein-ligand oleanolic acid bound to Kat G.

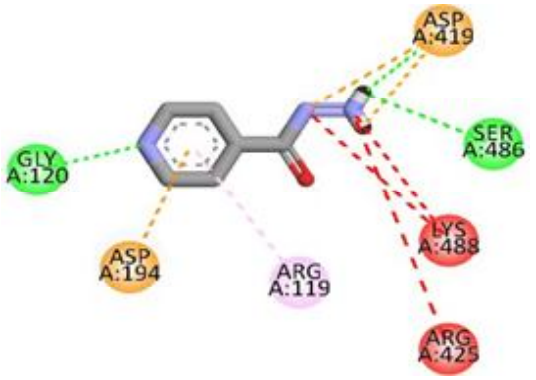


Figure 4. The 2D structure and interaction visualization of protein-ligand isoniazid bound to Kat G.

Figure 3. and Figure 4. shows the molecular interaction and binding site residues of the bioactive constituent and the control drug towards Kat G. Whereas H- bond are Tpr300(A), conventional hydrogen bond are Ala281(A), Van der waals are Ala139(A), Pro136(A), Pro286(A), Gln224(A) and Asn231(A), and a alkyl are Leu205(A), Leu227(A)and Pro232(A) which, shows the interaction between bioactive compound oleanolic acid and Kat G. H-bond are Gly120(A) and Ser486(A) attractive charges are Asp194(A) and Asp419(A), alkyl are Arg119(A) which, shows interaction between drug isoniazid and Kat G.

Molecular docking demonstrates the computational outcome through binding affinity scores which shows the validation of target protein and ligand. In addition, molecular docking also provides 3D structure visualization and the chemical interactions[1]. Molecular dynamic stimulation is most important process to determine protein-ligand stability and complex interactions and also to understand the conformational characteristics of docked complexes. Energy optimization for docked complex was performed using steepest descent algorithm. 50,000 steps were employed for energy optimization for each stimulation process[22]. In molecular dynamic stimulation used to found all parameters which includes H-Bond, RMSD, RMSF, SASA, and ROG by using the tool GROMACS.

In this study stimulation were carried out values between oleanolic acid and isoniazid against Kat G by Hydrogen bond (Figure 5), RMSD (Figure 6), RMSF (Figure 7), ROG (Figure 8), and SASA (Figure 9). Molecular dynamic stimulation not only predict but also it provides various biomolecular mechanism such as protein conformational integrity, binding of a ligand and molecular configuration changes and also occurs in time -resolved stimulation[15].

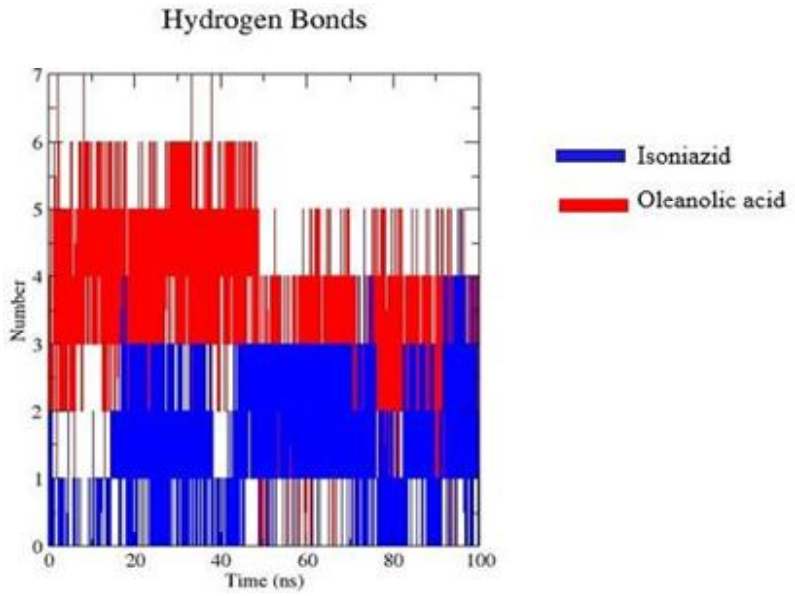


Figure 5. Molecular dynamic stimulation of Kat G – Hydrogen bond.

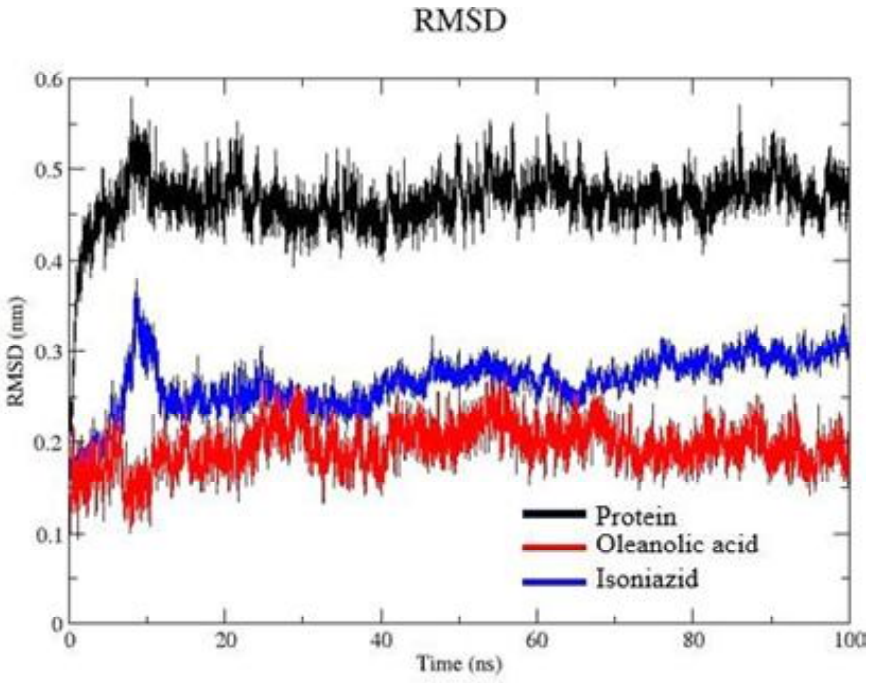


Figure 6. Molecular dynamic stimulation of Kat G – RMSD.

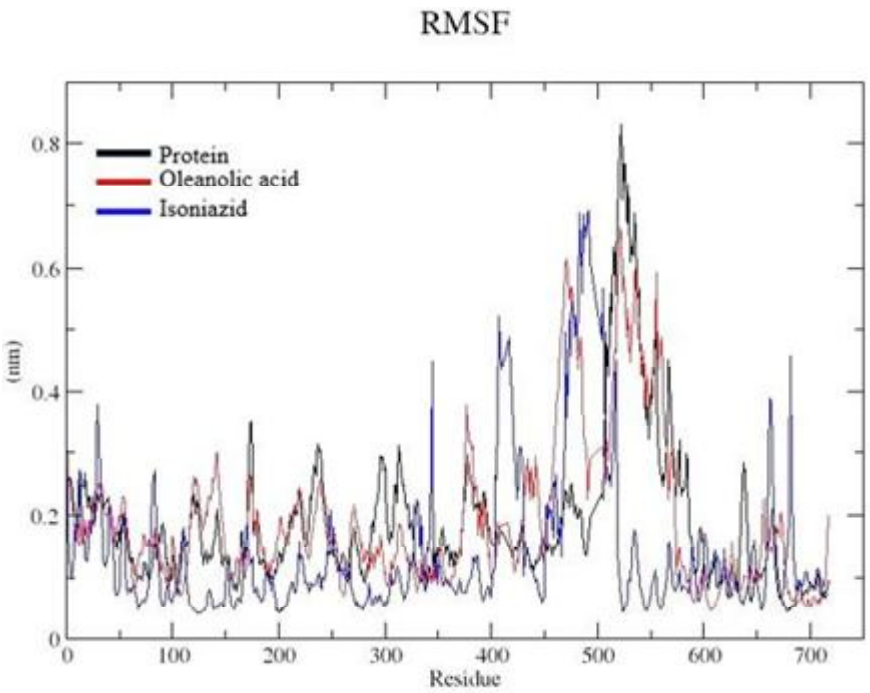


Figure 7. Molecular dynamic stimulation of Kat G – RMSF.

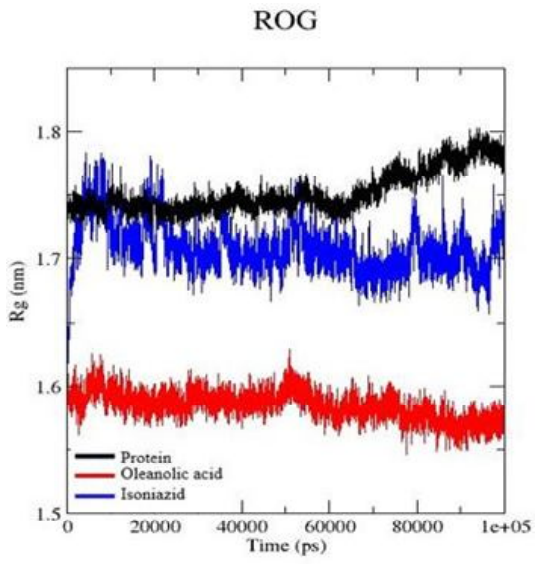


Figure 8. Molecular dynamics of Kat G –ROG

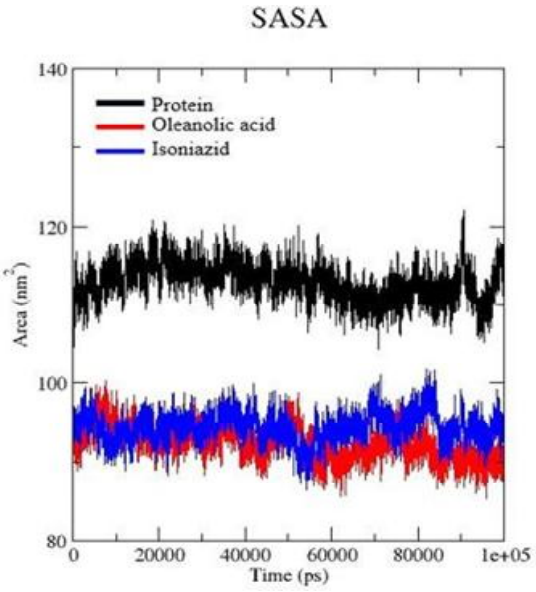


Figure 9. Molecular dynamics of Kat G SASA.

Naturally occurring phytoconstituent from *L. aspera*, in that Oleanolic acid has high binding affinity and which might have anti-tuberculosis activity. More significantly, by using computational approach including docking and molecular dynamic stimulation shows higher binding affinity as an anti-tuberculosis drug candidate through Kat G enzymatic suppression, as compared to control drug isoniazid. Overall, this study offers novel insight into identifying potential drug targets and potential agent against drug resistance Mycobacterium tuberculosis.

Authors' contributions

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Conflict of Interest

None.

References :

1. Abbas, M., A. R. Alanzi, K. I. Sahibzada, M. Nawaz, G. Fatima, and D. Q. Wei, (2025). *Scientific Reports*, *15*(1): 12795.
2. Agu, P.C., C.A. Afiukwa, O.U. Orji, E.M. Ezeh, I. H. Ofoke, C. O. Ogbu, ... and P.M. Aja (2023). *Scientific reports*, *13*(1): 13398.
3. Caminero, J.A., A. Matteelli and C. Lange, (2012). *European Respiratory Monograph*, *58:* 154-166.
4. Collins, H. L., U. E. Schaible, and S. H. Kaufmann, (1998). *The Journal of Immunology*, *161*(10): 5546-5554.
5. Dowdy, D. W., A.S. Azman, E. A. Kendall, and B. Mathema, (2014). *Clinical Infectious Diseases*, *59*(8): 1123-1129.
6. Gautam, S., K. A. Qureshi, S. B. Jameel Pasha, S. Dhanasekaran, A. Aspatwar, S. Parkkila, ... and D. Venugopal, (2023). *Antibiotics*, *12*(3): 541.
7. Goletti, D., G. Meintjes, B. B. Andrade, A. Zumla, and S. S. Lee, (2025). *International Journal of Infectious Diseases*, *150*.
8. Gulbay, B. E., O. U. Gurkan, O.A. Yıldız, Z. P. Onen, F. O. Erkekol, A. Baccıoglu, and T. Acıcan , (2006). *Respiratory medicine*, *100*(10): 1834-1842.
9. Heym, B., P. M. Alzari, N. Honore, and S.T. Cole (1995). *Molecular microbiology*, *15*(2): 235-245.
10. Heym, B., Y. Zhang, S. Poulet, D. Young, and S. T. Cole, (1993). *Journal of bacteriology*, *175*(13): 4255-4259.
11. Kumar, M., S.K. Singh, P. P. Singh, V. K. Singh, A. C. Rai, A. K. Srivastava, ... and A. Kumar, (2021). *Antioxidants*, *10*(12): 1990.
12. Lv, H., L. Wang, X. Zhang, C. Dang, F. Liu, X. Zhang, ... and Y. Xu, (2024). *Infectious Diseases of Poverty*, *13*(1): 70.
13. Mpeirwe, M., I. M. Taremwa, P. Orikiriza, P. E. Ogwang, D. Ssesazi, and J. Bazira, (2023). *Pharmacology & Pharmacy*, *14*(2): 33-42
14. Nagarasan, S. and M. Boominathan (2016). *Indo Am J Pharm Res*, *6*(09): 6567.
15. Putra, W.E., D. Widiastuti, A. Hidayatullah, M. F. Heikal, and A. M. R. Yusuf, (2024). *Science & Technology Asia*, 291-301.
16. Pym, A. S., B. Saint-Joanis, and S. T. Cole,

(2002). *Infection and immunity*, *70*(9): 4955-4960.
17. Rahman, M. S., S. K. Sadhu, and C. M. Hasan, (2007). *Fitoterapia*, *78*(7-8): 552-555.
18. Rampogu, S., B. Shaik, J. H. Kim, T. S. Jung, M. W. Ha, and K. W. Lee, (2023). *Heliyon*, *9*(2):
19. Sossen, B., M. Kubjane, and G. Meintjes, (2025). *International Journal of Infectious Diseases*, 107876.
20. Soujanya, K., B.A. Kumari and E. Jyothsna (2024). *Int J Adv Biochem Res*, *8*(2S): 504-508.
21. Suárez, I., S. M. Fünger, S. Kröger, J. Rademacher, G. Fätkenheuer, and J. Rybniker, (2019). *Deutsches Aerzteblatt International*, *116*(43):
22. Sundar, S., L. Thangamani, G. Manivel, P. Kumar, and S. Piramanayagam, (2019). *Informatics in Medicine Unlocked*, *16:* 100210.
23. Sungar, S., M. M. Toragall, M. Abdul Mujeeb, S. I. Puranik, A. A. Akbar, R. H. Aladkatti, and S. C. Ghagane, (2024). *Archives of Razi Institute*, *79*(4): 761.
24. World Health Organization, & Stop TB Initiative (World Health Organization). (2010). *Treatment of tuberculosis: guidelines*. World Health Organization.