Original Article

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Microbiology Section

Molecular Docking Study on Phytochemicals of *Alpinia galanga* and their Derivatives as Inhibitors of β-Ketoacyl Reductase (MabA) of *Mycobacterium tuberculosis*: An In-silico Study

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ABSTRACT

Introduction: Tuberculosis (TB), an infectious disease caused by the bacterium *Mycobacterium Tuberculosis* (MTB), continues to be a global health problem. *Alpinia galanga* (Linn.) of the Zingiberaceae family has antitubercular properties, and their mode of action in in-vitro as well as in-vivo conditions is well established. This knowledge of the active phytochemicals of *Alpinia galanga* has been utilised to identify new potent drugs for MTB.

Aim: To perform molecular docking studies of various phytochemicals of *Alpinia galanga* and their derivatives with β-ketoacyl reductase (MabA) of MTB.

Materials and Methods: The present study is an in-silico study conducted in the Bioinformatics facility of the Central Research Laboratory of Tagore Medical College and Hospital, Chennai, Tamil Nadu, India from November 2022 to April 2023. The receptor protein was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) database. The phytochemicals in. sdf format were downloaded from the PubChem database. The derivatives were prepared by Chemsketch software. Docking was performed using AutoDock Vina with PyRx as the GUI (Graphical User Interface). Post-docking analysis was performed in LigPlot+.

Results: Phytochemicals from *Alpinia galanga* were obtained from the PubChem database and docked with MabA of MTB. The derivatives were further subjected to docking analyses. From the docking study, two molecules, namely, (1E,6Z)-2,4-diamino-6-fluoro-1,7-bis(4-hydroxyphenyl)-1-sulfanylhepta-1,6-diene-3,5-dione and (2E,6Z,10E)-2,6,9,9-tetrakis(hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1), were found to have good binding energy values.

Conclusion: The present study helped us find drug-like molecules that can inhibit the MabA of MTB. Two compounds derived from the phytochemicals of *A. galanga* were found to have an effective binding capacity to the drug target in-silico. Hence, the outcome of present study has provided a therapeutic strategy for TB, especially for strains of MTB that are drug-resistant.

Keywords: Antimicrobial activity, Drug resistance, Mycolic acids

INTRODUCTION

Tuberculosis (TB), an infectious disease caused by the bacterium MTB, is one of the global health problems and continues to be a leading cause of morbidity and mortality [1,2]. India led the world in TB infections in 2022, accounting for a startling 27% of the worldwide burden, according to the World Health Organisation (WHO) worldwide TB report 2023. In total, 87% of all TB cases worldwide in 2022 were reported from 30 high burden TB countries, including Pakistan (5.7%), China (7.1%), Indonesia (10%), and the Philippines (7.0%) [3]. Studies have shown that *A. galanga* has antimicrobial activity; however, its active principles are yet to be explored. In the present study, molecular docking is used to identify drug candidates in the plant *A. galanga* with mycobacterial activity. Due to the increasing resistance to existing drugs, there is an urgent need for the development of new TB drugs [4,5].

Currently, the cell wall synthesis pathway of the bacteria is a promising target for new anti-TB drug discovery [6]. Mycolic acids are the major lipid components of the unique mycobacterial cell wall responsible for protecting the TB bacilli from external threats. Mycolic acids are synthesised in the cytoplasm and transported to the outer membrane. The large size of these unique fatty acids results of a huge metabolic investment that has been evolutionarily conserved, indicating the importance of these lipids for mycobacterial cellular survival. There are many key enzymes involved in the mycolic acid biosynthetic pathway, which are excellent potential drug targets, several of which show great promise as selective TB therapeutics [7].

During the biosynthesis of mycolic acids, in the second step of the elongation cycle, the resulting β-ketoacyl-ACP product is reduced by MabA, the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) dependent MabA of MTB [8]. Mycolic acids impart MTB with unique properties that defy medical treatment. They make the organism more resistant to chemical damage and dehydration, and limit the effectiveness of hydrophilic antibiotics and biocides [9]. Mycolic acids also allow the bacterium to grow inside macrophages, effectively hiding it from the host immune system. Mycolate biosynthesis is crucial for the survival and pathogenesis of MTB [10]. Thus, this enzyme is a potential drug target for the development of new drugs. Furthermore, the three-dimensional structure of the enzyme has been derived and is available in the protein database. This paves the way for molecular docking studies to identify lead molecules that can inhibit MabA.

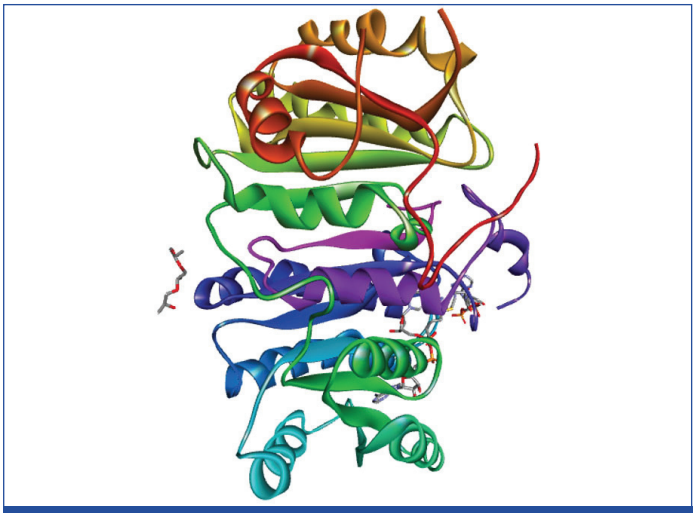
Alpinia galangal (L.) belongs to the family Zingiberaceae and is commonly referred to as galangal. It is widely cultivated in Southeast Asian countries, including India [11]. The plant is rich in phytochemicals with various pharmacological activities [12]. *A. galanga*, used in various traditional medicines, possesses broad-spectrum antibacterial properties [13]. *A. galanga* exhibits anti-TB

activity with multiple modes of action [14]. Since the activity of the extracts was observed under reducing oxygen concentrations, it may be effective in treating the dormant and non replicating bacteria of latent TB [14]. Studies have shown that the extracts of this plant have antimycobacterial activity against MDR strains of MTB [15,16]. Due to the emergence of multiple drug-resistant strains of MTB, there is a need for new drugs. Therefore, the aim of the present study is to perform molecular docking studies of various phytochemicals of *Alpinia galanga* and their derivatives with MabA of MTB to find promising drug candidates with anti-mycobacterial activity.

MATERIALS AND METHODS

The present study is an in-silico study conducted in the Bioinformatics facility of the Central Research Laboratory of Tagore Medical College and Hospital, Chennai, Tamil Nadu, India from November 2022 to April 2023.

Protein preparation: The present in-silico study involves the use of molecular docking software to simulate the interaction between target and drug-like molecules. The study was conducted in the Bioinformatics facility of the Central Research Laboratory of Tagore Medical College and Hospital. The three-dimensional structure of MabA [Table/Fig-1] of MTB was retrieved from the RCSB-PDB database through the search option (www.rcsb.org) and saved in the Protein Data Bank (PDB) format. The 3Diamensional (3D) structure of the protein was visualised in Discovery Studio software.



[Table/Fig-1]: Three-dimensional structure of β-ketoacyl reductase (MabA) of *M. Tuberculosis* (MTB).

Active site prediction: The possible binding sites of MabA are searched using the binding site prediction tool *3DLigandSite*, an online tool [17]. The best flexible binding sites were selected for this study.

Generation and optimisation of ligand: The structures of various flavonoids (quercetin, kaempferide, galangin) were obtained from the PubChem database. Their derivatives in 2D format were generated with the help of the ACD ChemSketch software [18]. The ligands were saved in mol 2 format. The Open Babel software was used to convert the mol format to pdb format [19]. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. A population size of 150 was set with 70 generations and one solution for quick docking. Based on the binding energy, the ligands were selected for further study [20]. The selected ligands were then analysed for drug-relevant properties based on Lipinski's rule of five (molecular weight <500 g/mol, not more than five hydrogen bond donors, not more than ten hydrogen bond acceptors, and a partition coefficient (log P) value <5). Other drug-like properties were analysed using the OSIRIS Property Explorer [21] and Molsoft: Drug-Likeness and Molecular Property Explorer [22]. On the basis of binding affinity and drug-like properties, the ligands were selected for further molecular docking studies.

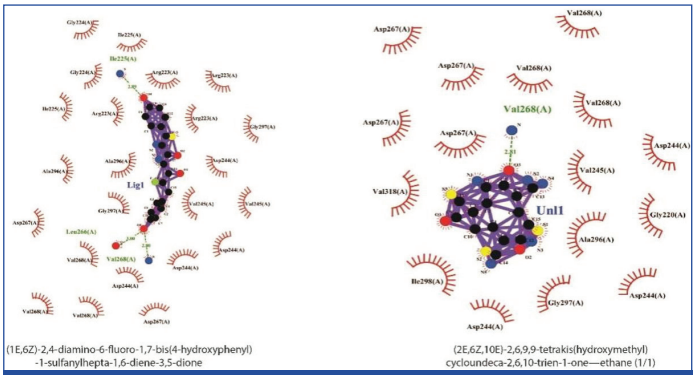
Protein-ligand docking: The docking of ligands was performed using AutoDock Vina software Vina, using PyRx as the GUI [23]. Docking was performed to obtain a population of possible conformations and orientations for the ligands at the binding site, along with their binding energy. Using the software, polar hydrogen atoms were added to the CTB and its non polar hydrogen atoms were merged. All bonds of the ligands were set to be rotatable. All calculations for protein-ligand flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. A grid box with dimensions of 126×126×126 points was used to cover the entire binding site and accommodate ligands to move freely. The best conformation was chosen based on the lowest docked energy after the docking search was completed.

RESULTS

The phytochemicals from *Alpinia galanga* were obtained from the PubChem database and docked with MabA of MTB. The best phytochemicals in terms of binding energy were utilised to prepare derivatives. The derivatives were further subjected to docking analyses. From the docking study, two molecules were found to have good binding energy values [Table/Fig-2]. The bound ligands were then subjected to LigPlot+ analysis to find the binding forces involved in it [Table/Fig-3].

Selected ligands	Binding energy kCal/mol	Interaction residues	Hydrogen bond	Hydrogen bond distance in Å
(1E,6Z)-2,4-diamino-6-fluoro-1,7-bis (4-hydroxyphenyl)-1-sulfanylhepta-1,6-diene-3,5-dione	-10.3	Gly 224, Ile225, Arg223. Gly297, Asp244, Val245, Asp267, Val268, Ala296	N-O Ile225 N-O Val268	2.89 2.80
(2E,6Z,10E)-2,6,9,9-tetrakis (hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1)	-10.2	Asp267, Val268, Val245, Asp244, Ala296, Gly220, Gly297, Ile298,Val318	N-O Val268	2.81

[Table/Fig-2]: Protein ligand interactions between the selected ligands.



[Table/Fig-3]: Docking analyses of the selected ligands with target protein.

The ligand (1E,6Z)-2,4-diamino-6-fluoro-1,7-bis(4-hydroxyphenyl)-1-sulfanylhepta-1,6-diene-3,5-dione showed two hydrogen bonds N-O Ile225 and N-O Val268 with bond lengths less than 3.00 Å. This shows to be an excellent tight binding of the ligand to the binding site of the drug target, potentially inhibiting it. Another ligand, (2E,6Z,10E)-2,6,9,9-tetrakis (hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1), showed a hydrogen bond N-O Val268 with a bond length less than 3.00 Å.

The selected two molecules underwent Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) studies in SwissADME and Chemicalise online server tools. Their drug likeness is excellent and fulfills many criteria of Absorption, Distribution, Metabolism and Excretion (ADME) [Table/Fig-4]. Both molecules obey the Lipinski's rule of five [Table/Fig-5].

Ligands	Bioavailability	Absorption		Metabolism		Excretion
		Intestinal absorption	Blood brain barrier absorption	CYP1A2 substrate	CYP3A4 substrate	P-glycoprotein substrate
(1E,6Z)-2,4-diamino-6-fluoro-1, 7-bis (4-hydroxyphenyl)-1-sulfanylhepta-1, 6-diene-3, 5-dione	0.55	low	No	Yes	Yes	No
(2E,6Z,10E)-2,6,9,9-tetrakis (hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1)	0.55	High	no	No	No	No

[Table/Fig-4]: ADMET properties of the derivatives.

Ligand	H-bond donors	H-bond acceptors	Molecular weight (Da)	Log P
(1E,6Z)-2,4-diamino-6-fluoro-1,7-bis(4-hydroxyphenyl)-1-sulfanylhepta-1,6-diene-3,5-dione	5	6	388.089	1.73
(2E,6Z,10E)-2,6,9,9-tetrakis(hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1)	4	5	282.146	0.42

[Table/Fig-5]: Lipinski's rule of five for the selected derivatives.

DISCUSSION

The treatment of TB has become a challenge in recent days due to the Multiple Drug Resistance (MDR) and Total Drug Resistance (TDR) strains of MTB [24]. Therefore, new drugs are needed to combat these strains. Molecular docking is a technique that can be exploited to develop new drugs for such strains [25]. Not many docking studies are conducted for the development of new drugs from plants for MTB [26]. Hence, in the present study, an attempt was done to identify drug-like molecules from the *A. galanga* plant that can inhibit MabA of MTB, an important drug target.

Molecular docking is least used technique in the identification of drug-like molecules from plants for bacterial diseases, especially MTB [27]. Many bacteria contain proteins that are crucial for their survival and pathogenicity [28]. Hence, they can be excellent drug targets for the generation of new drugs as alternatives to antibiotics [29]. These drugs will be target-specific, meaning they can inhibit or kill only a particular species or genus of bacteria.

The initial step of the study involved retrieving phytochemicals from *A. galanga* from the PubChem database, which serves as a comprehensive repository of chemical compounds. This approach allowed the researchers to efficiently screen a wide range of compounds and select those with potential therapeutic efficacy against MTB.

Subsequent molecular docking analyses were conducted to evaluate the binding interactions between the selected phytochemicals and the target enzyme, MabA. Molecular docking is a powerful computational technique used to predict the binding mode and affinity of small molecules with protein targets. In this study, the binding energy was used as a key parameter to assess the strength of interaction between the phytochemicals and the target enzyme. The lower the binding energy, the stronger the interaction between the ligand (phytochemical) and the receptor (MabA), indicating a higher likelihood of therapeutic efficacy.

Among the screened phytochemicals, those exhibiting the most favourable binding energies were chosen to design and synthesise derivatives. This rational design approach aimed to optimise the pharmacological properties of the lead compounds, enhancing their binding affinity and specificity towards the target enzyme.

The derivatives synthesised from the selected phytochemicals were then subjected to further docking analyses to evaluate their binding affinities. Interestingly, two molecules emerged from this analysis with particularly promising binding energy values. These molecules exhibited strong interactions with the target enzyme, suggesting their potential as potent inhibitors of MabA and, consequently, as anti-TB agents.

The study has revealed that certain phytochemicals and their derivatives do have the capacity to bind the drug target in-silico effectively. These molecules can be taken for further in-vitro and in-vivo studies to establish their ability to bind to the drug target. Production of such compounds is economical as they are derived from plant sources [30].

Overall, the results of present study highlight the therapeutic potential of phytochemicals derived from *A. galanga* against MTB. By employing computational approaches such as molecular docking, the study provides valuable insights into the molecular mechanisms underlying the interaction between the identified phytochemicals and the target enzyme. Further experimental validation, including in-vitro and in-vivo studies, will be necessary to confirm the efficacy and safety of these phytochemicals as anti-TB agents. Additionally, structural optimisation and medicinal chemistry efforts could be pursued to enhance the potency and selectivity of the identified lead compounds, ultimately contributing to the development of novel treatments for TB.

Limitation(s)

The study involves molecular docking, which is an *in-silico* study. It helps to shortlist and identify drug-like molecules. However, further high-throughput studies should be done to confirm the activity of the molecules confirmed by molecular docking.

CONCLUSION(S)

Tuberculosis is a dreadful disease and remains one of the top infectious diseases worldwide. Throughout history, various plants have been used for treating diseases and for commercial drug preparations. While extensive work is required to explore plant-based drugs traditionally, in-silico studies help in exploring new drugs from medicinal plants against many infectious diseases, reducing the need for animal experiments. The present study helped us find drug-like molecules that can inhibit MabA of MTB. Two compounds, namely (1E,6Z)-2,4-diamino-6-fluoro-1,7-bis(4-hydroxyphenyl)-1-sulfanylhepta-1,6-diene-3,5-dione and (2E,6Z,10E)-2,6,9,9-tetrakis (hydroxymethyl) cycloundeca-2,6,10-trien-1-one-ethane (1/1), were found to have effective binding capacity to the drug target in-silico. Since these molecules are identified from the phytochemicals present in the plant, they can be obtained from plants and used for further studies. Hence, the outcome of this study has provided a therapeutic strategy for TB, especially for the strains of MTB that are drug-resistant.

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REFERENCES

[1] De Backer AI, Mortelé KJ, De Keulenaer BL, Parizel PM. Tuberculosis: Epidemiology, manifestations, and the value of medical imaging in diagnosis. JBR-BTR. 2006;89(5):243-50.
[2] Hirsh AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM. Stable association between strains of Mycobacterium tuberculosis and their human host populations. Proc Natl Acad Sci USA. 2004;101(13):4871-76.
[3] World Health Organisation. Global tuberculosis report 2023. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023.
[4] Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. Totally drug-resistant tuberculosis in India. Clin Infect Dis. 2011;54(4):579-81.

[5] Dsouza DTB, Mistry NF, Vira TS, Dholakia Y, Hoffner S, Pasvol G, et al. High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the revised National tuberculosis control programme in an urban metropolis (Mumbai) in Western India. BMC Public Health. 2009;9:211.
[6] Anguru MR, Taduri AK, Bhoomireddy RD, Jojula M, Gunda SK. Novel drug targets for Mycobacterium tuberculosis: 2-heterostyrylbenzimidazoles as inhibitors of cell wall protein synthesis. Chem Cent J. 2017;11(1):68.
[7] North EJ, Jackson M, Lee RE. New approaches to target the mycolic acid biosynthesis pathway for the development of tuberculosis therapeutics. Curr Pharm Des. 2014;20(27):4357-78.
[8] Silva RG, de Carvalho LPS, Blanchard JS, Santos DS, Basso LA. Mycobacterium tuberculosis β-Ketoacyl-Acyl Carrier Protein (ACP) Reductase: Kinetic and chemical mechanisms. Biochemistry. 2006;45(43):13064-73.
[9] Lampart PA. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. J Appl Microbiol. 2002;92:46S-54S.
[10] Takayama K, Wang C, Besra GS. Pathway to synthesis and processing of mycolic acids in mycobacterium tuberculosis. Clin Microbiol Rev. 2005;18(1):81-101.
[11] Kaushik D, Yadav J, Kaushik P, Sacher D, Rani R. Current pharmacological and phytochemical studies of the plant Alpinia galanga. Zhong Xi Yi Jie He Xue Bao. 2011;9(10):1061-65.
[12] Chudiwal AK, Jain DP, Somani RS. Alpinia galanga Willd- An overview on phyto-pharmacological properties. J Nat Prod Resour. 2010;1(2):143-49.
[13] Tian Y, Jia X, Wang Q, Lu T, Deng G, Tian M, et al. Antioxidant, antibacterial, enzyme inhibitory, and anticancer activities and chemical composition of alpinia galanga flower essential oil. Pharmaceuticals (Basel). 2022;15(9):1069.
[14] Gupta P, Bhatter P, D'souza D, Tolani M, Daswani P, Tetali P, et al. Evaluating the anti Mycobacterium tuberculosis activity of Alpinia galanga (L.) Willd. Axenically under reducing oxygen conditions and in intracellular assays. BMC Complement Altern Med. 2014;14:84.
[15] Alajmi MF, Mothana RA, Al-Rehaily AJ, Khaled JM. Antimycobacterial activity and safety profile assessment of alpinia galanga and tinospora cordifolia. Evid Based Complement Alternat Med. 2018;2018:2934583.
[16] Basri AM, Taha H, Ahmad N. A review on the pharmacological activities and phytochemicals of Alpinia officinarum (Galangal) extracts derived from bioassay-guided fractionation and isolation. Pharmacognosy Reviews. 2017;11(21):43-56.
[17] Wass MN, Kelley LA, Sternberg MJ. 3D ligand site: Predicting ligand-binding sites using similar structures. Nucleic Acids Res. 2010;38:469-73.
[18] Advanced Chemistry Development, Inc. ACD/ChemSketch Freeware, version 10.00. Toronto, ON, Canada, 2012.
[19] O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. J Cheminform. 2011;3:33.
[20] Yang JM, Chen CC. GEMDOCK: A generic evolutionary method for molecular Docking. Proteins: Structure, Function, and Bioinformatics. 2004;55:288-304.
[21] Sander T. OSIRIS Property Explorer. Organic Chemistry Portal. Available from: https://www.organic-chemistry.org/prog/peo/.
[22] Walters WP, Murcko MA. Prediction of 'drug-likeness'. Adv Drug Deliv Rev. 2002;54(3):255-71.
[23] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-91.
[24] Jeon D. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update: Applicability in south Korea. Tuberc Respir Dis (Seoul). 2017;80(4):336-43.
[25] Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011;7(2):146-57.
[26] Pinto VS, Araújo JSC, Silva RC, da Costa GV, Cruz JN, De A Neto MF, et al. In-silico study to identify new antituberculosis molecules from natural sources by hierarchical virtual screening and molecular dynamics simulations. Pharmaceuticals (Basel). 2019; 12(1):E36.
[27] Pisano MB, Kumar A, Medda R, Gatto G, Pal R, Fais A, et al. Antibacterial activity and molecular docking studies of a selected series of hydroxy-3-arylcoumarins. Molecules. 2019;24(15):2815.
[28] Haag NL, Velk KK, Wu C. Potential antibacterial targets in bacterial central metabolism. Int J Adv Life Sci. 2012;4(1-2):21-32.
[29] Cattoir V, Felden B. Future antibacterial strategies: From basic concepts to clinical challenges. J Infect Dis. 2019;220:350-60.
[30] Smith AB, Johnson CD, Brown EF. Phytochemicals and their derivatives as potential inhibitors of drug targets in tuberculosis: In-silico binding studies. J Med Chem. 2023;10(3):215-20.

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