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Research Article
In silico Studies for Potential Natural Inhibitors for Isocitrate Dehydrogenase Type II of *Mycobacterium tuberculosis* (H37Rv)

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Abstract

Background and Objective: Tuberculosis is a life threatening bacterial disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) which has affected the population of almost all parts of the world since time immemorial. Although remarkable discovery has been achieved in combating disease control and its propagation but due to emergence of resistant strain of *M. tuberculosis* exhaustive search for the panacea to counter the bacteria is still on. This study proposes *in silico* studies of 35 natural compounds against proposed drug target isocitrate dehydrogenase type II (PDBID: 5 KVU) of *M. tuberculosis* (H37Rv). Almost all available antitubercular drugs in the market now-a-days have some side effects. Hence, there is a strong need of some natural antitubercular compounds that can mitigate the side effects and boost up the overall health of the patients. The aim of the present study was to explore the potential novel natural inhibitor against proposed drug target, isocitrate dehydrogenase II. **Materials and Methods:** Crystal structure of isocitrate dehydrogenase II was obtained from PDB. All natural compounds used in this study were obtained from Pubchem database and their drug likeliness was also crosschecked. Docking studies were performed with the help of Autodock 4.0. $NADP^+$ was used as a reference ligand to compare the docking results with natural compounds. Molecular dynamics study of the docked complex has also been performed to infer the deep insight of the various interactions and stability of the receptor-ligand docked complex. **Results:** Results showed that molecular docking of isocitrate dehydrogenase II of *M. tuberculosis* against all 35 natural multi-beneficial compounds, Amentoflavone attained the minimum binding energy, hence amentoflavone is most potential natural compound that may inhibit the proposed enzyme. The molecular dynamics study of the docked receptor ligand complex also showed good congruence to the docking result. **Conclusion:** Natural compounds showed good binding energies with the receptor protein isocitrate dihydogenase II and amentoflavone, a natural ligand may act as a strong anti-tubercular lead compound against *M. tuberculosis*.

Key words: *Mycobacterium tuberculosis*, isocitrate dehydrogenase II, molecular docking, natural ligands, molecular dynamics

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tuberculosis (TB) has been one of the global major health problems since ages and this disease is responsible for death of millions of people and the cases are mostly from the developing countries. *Mycobacterium tuberculosis* is a causative agent of tuberculosis disease. According to World Health Organization (WHO), TB report developing countries are hotspot carrying about 95% reported cases, of which 25% infected people met death. India has been listed among the countries which are carrying a high burden of the disease and having incidence rate of Multi-drug Resistant Tuberculosis (MDR TB) 9.9%[1,2]. In the present scenario, the most promising anti-TB drugs targets are from information-pathways (DNA and RNA polymerases or DNA gyrase)[3]. On the other hand, the metabolic pathways which are unique to the pathogen might be chosen as a drug target because of their essentiality and vitality for the survival of the organism. Tricarboxylic acid cycle (TCA) is one of the key metabolic pathway of *M. tuberculosis*. The *M. tuberculosis* genome is annotated to encode a complete TCA cycle[4,5]. Isocitrate dehydrogenase is one of most important enzymes of the citric acid cycle, that catalyses oxidative decarboxylation. *Mycobacterium tuberculosis* isocitrate dehydrogenase-II (mtbicd2) is $NADP^+$ dependent enzyme, having no similarity with human isocitrate dehydrogenase[6]. The citric acid cycle is essential for the perseverance and survival of the organism. The mtbicd2 has also been proven as a potential molecular drug target[7]. Annotation and essentiality of enzyme mtbicd2 (Rv0066) was cross checked from Tuberculist database[8].

The aim of the present study was to explore the potential novel natural inhibitor against proposed drug target, isocitrate dehydrogenase II. This enzyme may act as a potential target against mtb as it is a key enzyme of citric acid cycle and it's inhibition may inhibit the growth of bacteria. In present study, the chemical compounds which are well known for their benefits were taken instead of going for random library screening, so these compounds do not have any known reactive or toxic effects that enhances the quality of probable anti-tubercular compound among them. The emergence of resistant strain of *Mycobacterium* and side effects of anti-tubercular drugs have made a necessity of the time to go for new targets as well as naturopathy to treat this deadly bacteria. This study showed a comparative analysis with the help of molecular docking for proposing the best possible natural inhibitor for mtbicd2 among 35 natural (Table 1) occurring compounds. The $NADP^+$ is natural ligand that is responsible for the activity of the isocitrate dehydrogense II. Molecular docking was done also with $NADP^+$ against isocitrate dehydrogenase II at same binding site. Most of the natural ligands showed lower binding energy than $NADP^+$. So it could be proposed that these ligands may bind efficiently with enzyme replacing $NADP^+$ and hence block the activity of the enzyme. Amentoflavone showed the lowest binding energy thus, could distort enzyme's function by binding and inhibiting it.

MATERIALS AND METHODS

Present study was carried out at Interdisciplinary Institute of Science, University of Allahabad, India in October-November, 2017.

Preparation of the protein: The protein selected as a drug target for the study was mtbicd2 (PDB ID: 5KVU) whose 3D structure was obtained from protein data bank (https://www.rcsb.org). Water molecules and hetero-molecules were removed from the crystal structure of the receptor protein and polar hydrogen and charges were added to the protein for molecular docking. The fpocket programme was used for pocket detection[9]. The amino acids Asn87, Ser134, Asn137, Tyr422, Asp550, Asp551, Ser565, Gly587, Ser588, Ile350, Arg141, Arg652, Ala589, Pro590 and His592 were found to present in the active site of proposed target protein (5KVU).

Preparation of ligand: The structures of all 35 natural compounds were downloaded from PubChem database in SDF format[10]. These natural compounds were converted into PDB file with the help of Open Babel software[11], widely used for the interconversion of many chemical file formats.

Molecular docking with Autodock4.0: Identification of the binding modes and conformation of ligands in the active site was done using Lamarckian genetic algorithm of Autodock4.0[12]. Heteroatoms and water molecules were removed from the crystal structure of the protein and polar hydrogen and charges were added to the macromolecule during the protein preparation. For performing the flexible docking ligands torsion was made rotatable. Grid maps were taken around the active site of the target by using Autogrid with a grid-point spacing of 0.369 and grid box size $55\times60\times55$. Molecular Docking was done using Lamarckian genetic algorithm with a population size of 150. Docked conformations were obtained with a mutation rate of 0.02, population size of 150 and crossover rate of 0.8. The H-bonds formed, residues involved in forming H-bonds and interactions between ligand and target protein were analyzed in pymol (http://pymol.org).

Table 1: This table describes the compounds selected for the docking studies with their Name, Pubchem Id and their class, the minimum energy obtained after docking against enzyme (mtbicd2 PubChem), H-bonds formed with involved and interacting amino acid residues(one letter code) of the enzyme

Compound name, PubChem CID and class	Binding energy (Kcal mol^{-1})	Number of H bonds formed/involved residues	Interacting residues
Amentoflavone, CID 5281600, Flavonoid	-9.77	4, S134, Q94, Q94, N137	S89, N87, Y422, I88, G587, Y422, R141, S588, R652
Diospyrin, CID 308140, Bisnaphthoquinone	-9.30	2, G587, R147	R147, N137, R141, Q94, A555, D352, S89, A585, N87
Stigmasterol, CID 5280794, Phytosterol	-9.20	3, R550, R141, Y422	S89, Q94, S588, P590, S347, D348, I350, D551, R550, G587
Obtusifoliol, CID 65252, Phytosterol	-7.99	2, S588, R141	I88, R147, Q94, D352, P590, R550, D348, Y422, D348, A551
Caniojane, CID 101239632, Diterpene	-7.93	3, Q94, D348, K257	A353, I350, G87, S588, P590, S89, I88, D348,S347, Q94, S59
Demethoxycurcumin, CID 5469424, Curcuminoids	-7.76	3, R141, K257, N87	G587, Y422, Ie350, P590, N137, A589, G587, R141, Q94, L85
Oleanolic acid, CID 10494, Flavonoid	-7.59	No H bonds formed	S89, D551, S347, Y422, I350, Y422, G588, N137
LicarinaA, CID 5281836, Phenylpropanoids	-7.40	2, R550, N87	L85, G586, G584, P86, R147, R550, R141, N137, K257, D551, K84y6
Andrographolide, 5318517, Flavonoid	-7.34	3, R550, G587, S588	S89, D352, R147, D555, P590, N137, A589, Y422, I350
Ursolic acid, CID 64945, Flavonoid	-7.28	2, N137, D550	I350, Y422, G588, N137, S89, D551, S347, R141, I88, Q94, D551
Grayanin, CID 6442904,Cyanogenic Glucosides	-7.10	5, N87, R141, D550, S89, Y422	P590, R550, D348, R147, D555, P590, Q94, L85
Stipulin, CID 10069091, Flavonoid	-7.05	3, N87, T259, G584,	K257, N187, T259, S89, N137, R141, G586, L85, G587
Kanzonol C, CID 5316802, Flavonoid	-6.97	2, D555, S89	G586, P86, T hr259, S89, N137, R141, E582, G584, P86, I88, I350
Ferruginol, CID 442027, Flavonoid	-6.86	1, N87	R141, N137, E582, A585, K257, N187, G584, G586, P86, I85, I350
Kaurene, CID 520687, Flavonoid	-6.74	No H bonds formed	P86, N87, A353, D352, D551, D555, N137, S565, P86I87
Cryptocaryon, CID 42607660, Dihydrochalcones	-6.71	1, S89	R550, D551, A585, D555, I350, N87, S588, A589, Y422, S134
Piperine, CID 638024, Alkaloid	-6.64	1, S89	T259, S89, N137, R141, E582, G584, P86, I88, N87, G586, L85, G587
Tiliacorine, CID 20055053, Alkaloid	-6.41	No H bonds formed	Q84, T259, Y422, N137, S84, S89, R141, P590, N137, R555, S588
Pinocembrin, CID 68071, Flavonoid	-6.39	4, N137, N87, G586, G584	N137, K84, S84, N187, R141, N137, N87, K257, S585
Nummularine F, CID 5281594, Alkaloid	-6.38	No H bonds formed	L85, N137, I85, N137, P86, K257, D352, A585, G586
Aristolactum, CID 96710, Alkaloid	-6.82cc	3, S134, R141, R550	K257, Met260, S134, S89, N137, R141, K84, L85, D551
4-Epi-larreatricin, CID 11033399, Diterpenes	-6.30	1, G 584	N87, S134, N137, R603, P590, A589, Y422, R141
Globiferin, CID 44140144, Monoterpenes	-6.30	1, N87	D555, D551, P590, R141, A353, I350, T259, G587, Q94, S347
Isothymusin, CID 630253, Flavonoid	-6.28	2, N87, D352	I350, K257, D352, N137, N187, K84, L85, E582, R141, A585
Plumericin, CID 5281545, Monoterpenes	-6.25	5, S89, R141, R147, K257, R550	R141, Met260, K257, T259, S89, S134, R550, Y422, D551, R147, A585
Paromomycin, CID 196959, Anteaglonialides	-6.24	1, N87	K257, A585, R141, N137, G584, Q582, K84, N87, I350
Maritinone, CID 633024, Quinones	-6.77	1, R550	D352, S134, N87, S89, N137, R141, R550, T259, Y422, T259
Naringenin, CID 932, Flavonoid	-6.14	4, N87, 137, Y422, G558	G586, P86, E582, L85, K584, R141, G587, A589, A585, G586
Pisonin, CID 53356511, Chromone	-6.08	4, R141, R147, K257, R550	K257, Met260, D352, D551, D555, R550, R141, A585
Eupomatenoid 5, CID 6443783, Neolignans	-6.04	3, Y433, R550, R141	D555, D551, D352, Y422, S89, P590, R141, G587, A589
Prunetin, CID 5281804, Flavonoid	5.95	2, D352, G586	I350, K257, D352, A585, E582, N137, N87, K584, G584, E582, A585, G586
Genistin, CID 5281377, Flavonoid	-5.86	3, N87, D352, G584	R141, A585, G587, N87, A589, A353, D352, I350, D555, N137
Nevadensin, CID 160921, Flavonoid	-5.78	3, R141, R147, R550	R147, R550, R141, N137, D555, D551, Y422, K257, D352, S585
NADP, CID 5886, Coenzyme(reference ligand)	-5.35	6, S89, R147, K257, D352, R550, D555, D551	N87, R652, D550, G587, S588, R652, R603, A589, His592, P590
Mauritine D, CID 102146051, Alkaloid	-5.42	1, A585	D551, D555, D352, A353, K257, Met260, S134, S89, N137, P86, S565, G586
Curcumene, CID 92139, Curcuminoids	-5.28	No H bonds formed	R147, R550, D551, A585, G586, P86, E582, L85, K584, N137, R141, Y422

Molecular dynamics simulation: Molecular dynamics simulation was performed by using Gromacs 4.5.3 package[13] on Intel Xeon Quad Core machine with 6 GB RAM CentOS 7 Linux package. The Root Mean Square Deviation (RMSD), Root Mean Square Fluctuations (RMSF), Solvent Accessible Surface Area (SASA) and number of H-bond variations, protein-ligand complex structure was studied. The RMSD was commonly used as an indicator of convergence of the structure towards an equilibrium state. This gives insight into the flexibility of regions of the protein and corresponds to the crystallographic b-factors (temperature factors). Radius of gyration was also calculated which gave an indication of the shape of the molecule at each time. Structure of docked protein complex was used as starting point for molecular dynamic (MD) simulations. Systems were solvated in a cubic box with simple point charge (SPC) water molecules at 10 angstrom marginal radius. At physiological pH, the structures were found to be negatively charged, thus in order to make the simulation system electrically neutral, 26 sodium ions (Na^+) were added to the simulation box. Initially the solvent molecules were relaxed while all the solute atoms were harmonically restrained to their original positions with a force constant of 10 kcal mol^{-1} for 5000 steps. After this, whole molecular system was subjected to energy minimization for 50000 iterations by steepest descent algorithm. Berendsen temperature coupling method was used to regulate the temperature inside the box. Electrostatic interactions were computed using the Particle Mesh Ewald (PME) method. Two small equilibration phases of normal volume and temperature (NVT) followed by normal pressure and temperature (NPT) was performed for 100 ps (picoseconds) each to properly relax the complex. The temperature was maintained at 300 K and pressure was maintained at 1 atm with the allowed compressibility range of atm. The SHAKE algorithm was used to constrain bond lengths involving hydrogen, permitting a time step of 2 fs (femtosecond) vander waals and coulomb interactions were truncated at 1.0 nm. The non-bonded pair list was updated every 10 steps and conformations were stored every 0.5 ps. Position restraint simulation for 500 ps was implemented to allow solvent molecules to enter the cavity region of structure. Finally, systems were subjected to (MD) simulation for 30 ns (nanosecond). Number of distinct hydrogen bonds formed between amino acid residues in protein complex were also analyzed so that we can estimate the stability of the complex can be estimated. The NH bond determined on the basis of donor-acceptor distance smaller than 0.35 nm and of donor-hydrogen-acceptor. All the graphs were plotted using XMGRACE program (http://plasma-gate.weizmann.ac.il/Grace/).

RESULTS

Molecular docking of natural compounds to the receptor target protein mtbicd2: The natural compounds taken for the molecular docking study with the proposed target mtbicd2 revealed the mode of action and process of the binding. All natural compounds (with name, PubChem CID and class), binding energy, number of H bonds formed, residues involved forming H-bonds and interacting residues with ligand has been given in the Table 1.

The mtbicd2 is dependent on $NADP^+$ for its enzymatic function, so $NADP^+$ is its natural ligand. Docking was also performed for $NADP^+$ with the target mtbicd2 and its resultant binding energy was compared with the binding energy of all 35 natural compounds. The results depicted that some natural compounds were showing lower binding energies (-6.45 to -10.28 kcal mol^{-1}) than $NADP^+$ (binding energy of -5.35 kcal mol^{-1}).

Amino acid Asn87, Gln94, Asp550, Ser588, Arg141, Lys257, Ser89, Asn137, Ala589 and Gly587 are responsible for H-bonds in all docking cases. Among all natural ligands amentoflavone, have lowest binding energy -9.77 kcal mol^{-1} and form 4 H-bonds with target. Thus, amentoflavone was chosen as the best potent compound against proposed target mtbicd2.

Amentoflavone, CID 5281600 (Fig. 1) ranked first among all the compounds on the basis of it's binding energy -9.77 kcal mol^{-1} and formation of four H-bonds with 11 hydrophobic interactions and 7 lipophilic interactions in docked complex with mtbicd2 (Fig. 2a, b). Amentoflavone is a naturally occurring biflavonoid that is extracted from

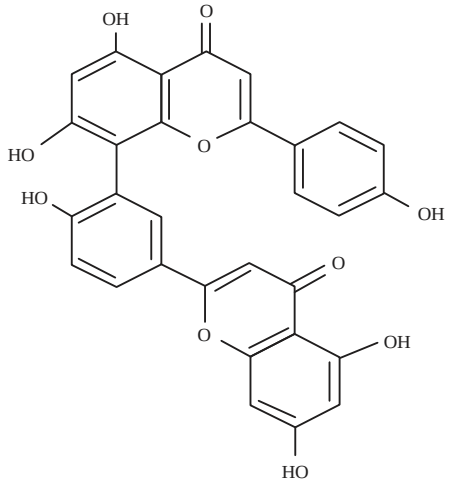

Fig. 1: Chemical structure of amentoflavone (PubChem CID 5281600)

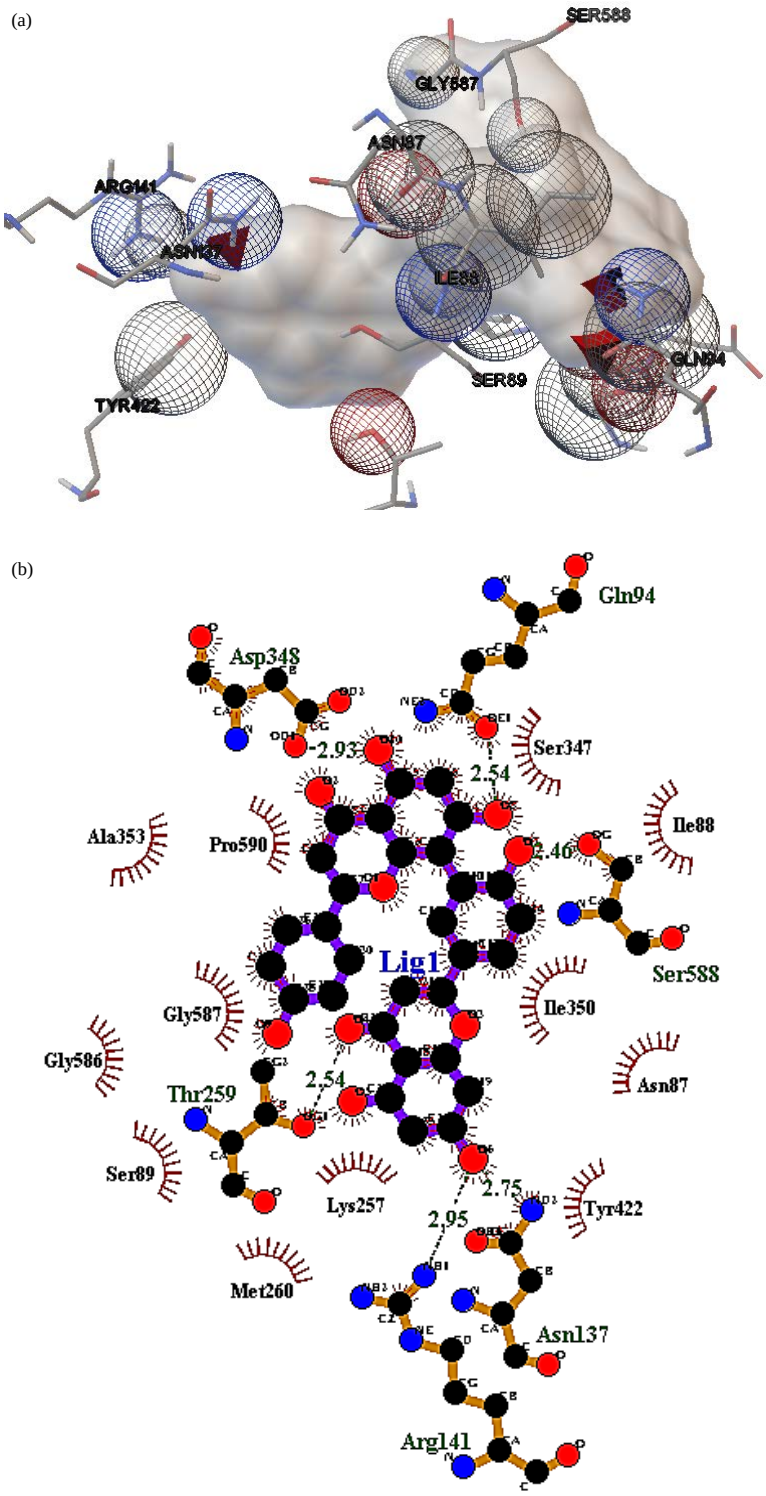

Fig. 2(a-b): (a) Interactions between ligand amentoflavone (solid surface, grey color) and receptor protein residues (spheres and lines) indicating residues name. The protruding triangles in red colour indicating intermolecular H bonds formed and (b) Representation of interactions between ligand amentoflavone and receptor protein by LigPlus. The H bonds are represented olive color dotted lines. The red spokes inwards to Ligand (Lig1) represents hydrophobic interactions formed between ligand and receptor protein

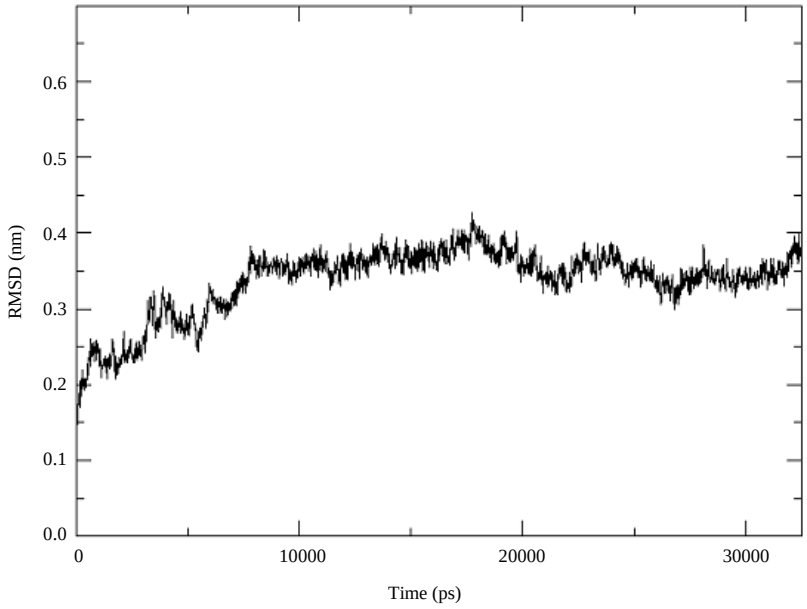

Fig. 3: RMSD of protein backbone with respect to time

the plant *Ginkgo biloba*, *Chamaecyparis obtusa* (hinoki), *Hypericum perforatum* and *Xerophyta plicata*. Amentoflavone is known as a mood enhancer and its potential to activate the GABA-A receptor and it is also used for the weight loss and body building purposes. This compound is also known for its anti-inflammatory, antineoplastic and antimalarial activities[14].

Molecular dynamics studies for *Mtbicd2* docked with amentoflavone: The RMSD, RMSF, radius of gyration (Rg), SASA and number of H-bond variations were studied. The RMSD was commonly used as an indicator of convergence of the structure towards an equilibrium state. The RMSD is merely a distance measure which is considered to be the most meaningful for low values. In Fig. 3, complex structures showed deviation till 10000 ps from their starting structure, resulting in a backbone RMSD of ~0.14-0.35 nm during the simulations. After this, native structure retained the maximum deviation till the end of the simulation resulting in the backbone RMSD of ~0.35-0.42 nm, respectively. The RMSD increased to a plateau value initially, this meant that the structure of the protein reached a certain distance from the reference structure and then kept that distance more or less same with the reference structure.

The RMSF captures, for each atom, the fluctuation about its average position. This gives insight into the flexibility of regions of the protein and corresponds to the crystallographic b-factors (temperature factors). Usually, one would expect similar profiles for the RMSF and the b-factors and this could be used to investigate whether the simulation results are in accordance with the crystal structure or not. The graph elaborates maximum fluctuation at both the C and N terminal of the protein which is very natural. The fluctuation range of backbone residue was in the range of ~0.1-0.5 nm (Fig. 4).

The radius of gyration was also calculated which gives an indication of the shape of the molecule at each time. The radius of gyration was compared to the experimentally obtainable hydrodynamic radius. This also shows the distribution of mass along the central axis i.e., backbone. In present study, it is clear that at starting stage it was higher in the range of 2.73 nm but continuously decreases to 2.6 nm in 8.0 ns and remains stable throughout the rest of the stages of the dynamics (Fig. 5). This infers that the protein-complex was a little bit less compact at the initial state but attained higher compactness during simulation.

One property which can be of interest is the surface area of the protein which is accessible to solvent, commonly referred to as the solvent accessible surface (SAS) or the solvent accessible surface area (SASA). This could be further divided into a hydrophilic SAS and a hydrophobic SAS. In addition, the SAS could be used together with some empirical parameters to obtain an estimate for the free energy of solvation. Graph of solvent accessible surface showed same pattern as radius of gyration as initially it was high (380 nm^2) and continually lower down to 355 nm^2 in 0.8 ns and remain constant throughout the dynamics (Fig. 6).

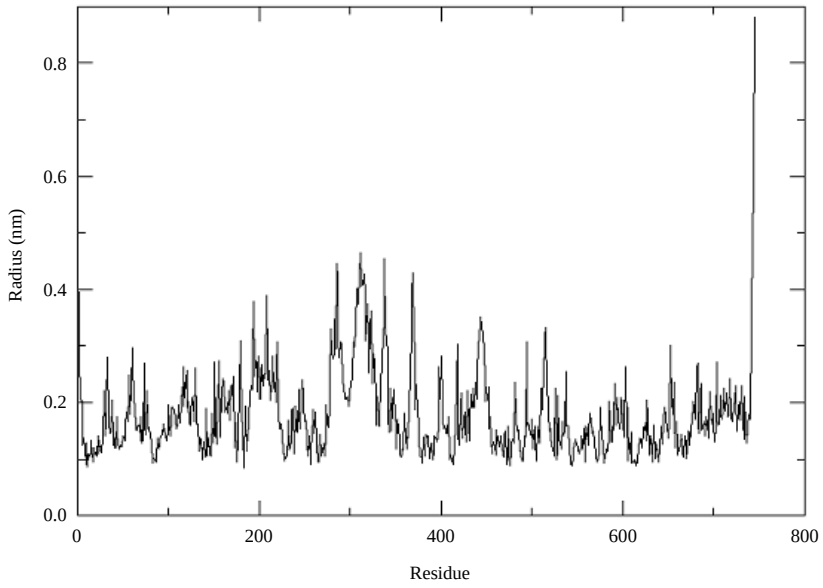

Fig. 4: RMS fluctuation graph with respect to residue number

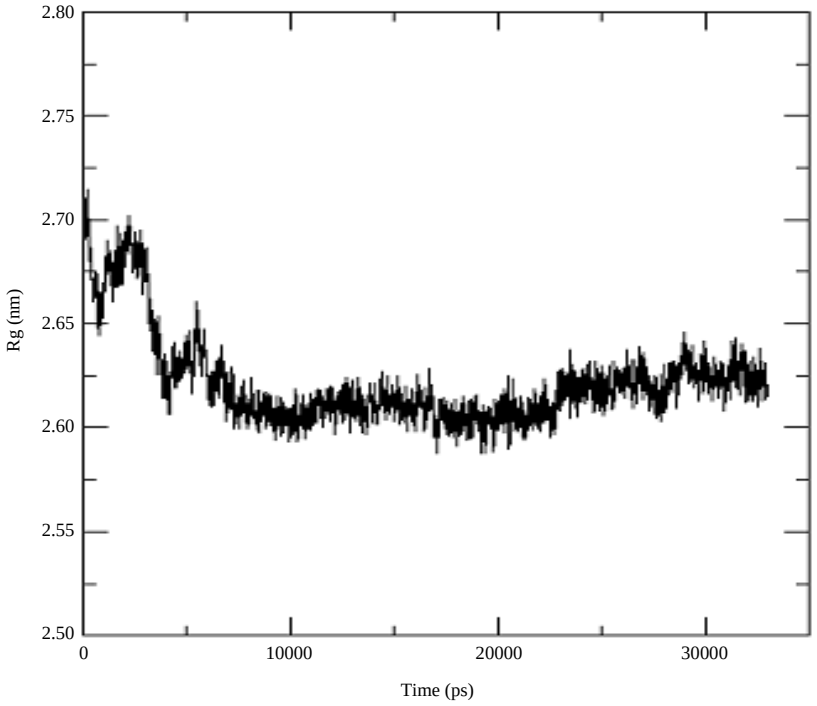

Fig. 5: Radius of gyration graph with respect to time

The stability of the secondary structure of protein by analyzing the number of hydrogen bonds, internally (protein-protein) were investigated. The presence of a hydrogen bond was inferred from the distance between a donor-H-acceptor pair and the donor-H-acceptor angle. The result produced in this simulation showed that number of internal hydrogen bonds increased initially from less than 530 and averaged over to ~600 during the entire dynamics (Fig. 7). The number of hydrogen bonds were directly associated with the stability of protein-complex and increase in the number support this assumption of stable MD simulation of protein-complex.

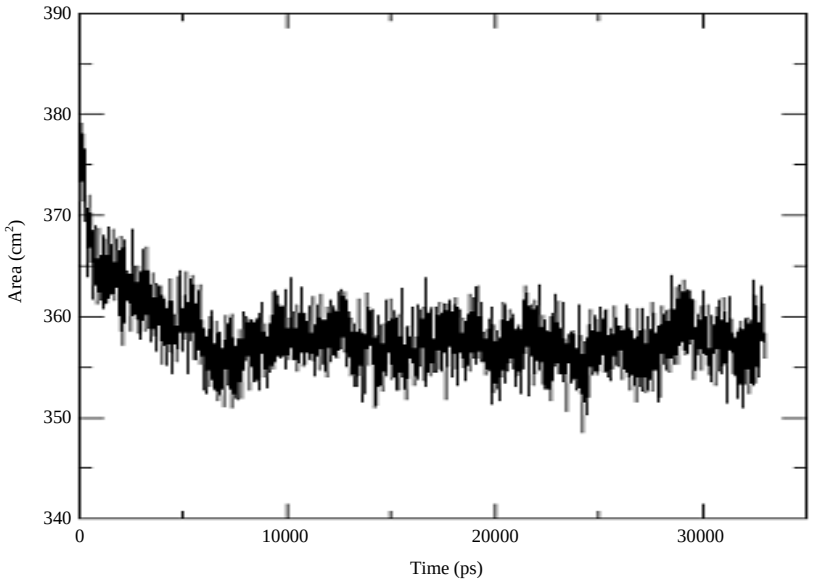

Fig. 6: Solvent accessible surface area graph with respect to time

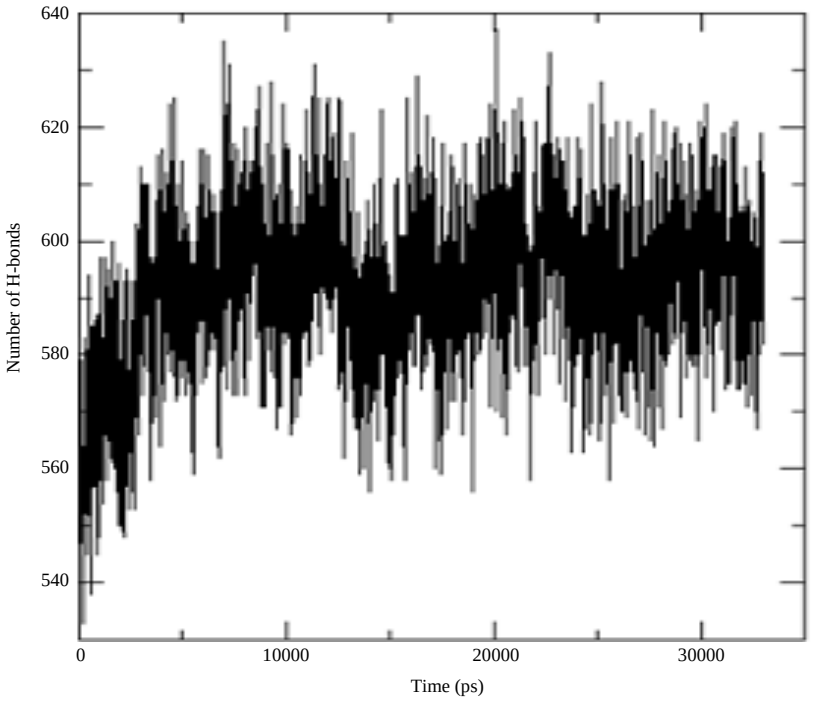

Fig. 7: Number of Intra-atomic H-bonds graph with respect to time

DISCUSSION

The current study showed that isocitrate dehydrogenase II mat act as a potential drug target against *M. tuberculosis* and investigated 35 natural compounds may act as potential inhibitors for crucial target isocitrate dehydrogenase II, which in turn check survival of *M. tuberculosis*. Among 35 natural compound investigated in this study, amentoflavone, which is also known for it's antimalarial and anticancer activity showed minimum binding energy (-9.77 kcal mol^{-1}) with the formation of 4 H-bonds and also showed a fair stability within the complex in molecular dynamics studies also. Thus, amentoflavone may act as a potential lead for the clinical trial.

Most of available first line anti-tuberculosis drugs in the market have a wide array of side effects. Earlier, some studies also proposed natural inhibitors against *M. tuberculosis* either computationally or by wet lab methods. Novel natural inhibitors for *M. tuberculosis* isocitrate lyase enzyme, which is also a enzyme of glyoxylate cycle, a variation of TCA cycle, were also proposed by virtual screening and molecular dynamics simulation[15]. In this way it was found that, nowadays metabolic pathway enzymes are proposed as a potential drug target against bacteria because bacteria play a crucial role for the survival of organisms and are needed with minimal level. In another study natural inhibitor from Columbian plant was extracted against MurE ligase enzyme of *M. tuberculosis*[16]. A review study for natural growth inhibitors for tuberculosis was done that proposes different class of compounds[17]. In a study some marine natural products from Turkish sponge *Agelas oroides* were proposed that inhibits Noyl reductases from *Plasmodium falciparum*, *M. tuberculosis* and *Escherichia coli*[18]. Therefore, development of new anti-tubercular compounds like herbal medicines is crucial for overall health benefits and treatment. Each and every natural compound which have been used in this study have medicinal significance and therapeutic effects[14,19-24].

CONCLUSION

Emergence and propagation of resistant strain of *M. tuberculosis* makes it difficult to control and cure tuberculosis. This study proposed mtbicd2 as potential drug target and some natural beneficial compounds that are specific inhibitors of mtbicd2. Molecular docking and molecular dynamics simulation studies explain the stability and fairly good interactions of target enzyme with amentoflavone, among all studied natural compounds.

Amentoflavone may be potential lead multi-beneficial antituberculosis compound that can be beneficial for tuberculosis treatment and health recovery.

SIGNIFICANCE STATEMENT

The study proposed mtbicd2 as potential drug target and some natural beneficial compounds that are specific inhibitors of mtbicd2 and revealed that amentoflavone among all compounds is most effective in interaction to target enzyme. This study opens new avenues for researchers to examine amentoflavone as potential leads in clinical trials and *in vivo* studies. Being a natural compound, this also nullifies the chances of adverse effect on human health and there are also least chances of developing resistant by *M. tuberculosis* as in case of using antibiotics.

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