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Research Article
Identification of Mycobacterial RNA Polymerase Inhibitors from the Main Phytochemicals of *Nigella sativa*: An *in silico* Study

[1]Shabir Ahmad Mir, [1,2]Mohammed Alaidarous, [1]Bader Alshehri, [3,4]Bilal Ahmad Mir, [1]Abdul Aziz Bin Dukhyil, [1,2,5]Saeed Banawas, [6]Ahmad Firoz, [1]Suliman A. Alsagaby, [1]Yahya Awaji Madkhali, [1]Sadaf Jahan, [7]Faizan Kashoo, [1]Danish Iqbal, [8]Showkat Ahmad Ganaie, [1]Ranjay Kumar Choudhary, [1]Kamal Shaker Albenasy and [1]Asma Naseem

[1]Department of Medical Laboratory Sciences, College of Applied Medical Science, Majmaah University, Al Majmaah 11952, Saudi Arabia
[2]Health and Basic Sciences Research Center, Majmaah University, Al Majmaah 11952, Saudi Arabia
[3]Department of Botany, School of Life Sciences, Satellite Campus Kargil, University of Kashmir, Kargil, Jammu and Kashmir 194103, India
[4]Department of Botany, Kargil Campus, University of Ladakh, Khumbathang, Kargil 194105, Ladakh, India
[5]Department of Biomedical Sciences, Oregon State University, Corvallis Oregon 97331, United States of America
[6]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[7]Department of Physical Therapy and Health Rehabilitation, College of Applied Medical Sciences, Majmaah University, Al Majmaah 11952, Saudi Arabia
[8]Department of Clinical Biochemistry, University of Kashmir, Jammu and Kashmir 190006, India

Abstract

Background and Objective: Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the major global public health concerns. Considering the limitations of the current anti-TB chemotherapy and due to the emergence of drug-resistant *M. tuberculosis* strains, there is an urgent need to discover and/or develop new drugs against TB. Plants and their extracts have been used in therapeutics since ancient times and their therapeutic importance is being increasingly explored in recent times. In this study, we screened the major phytochemicals of the plant, *Nigella sativa* for assessing their potential to inhibit the RNA polymerase (RNAp) of *M. tuberculosis*. **Materials and Methods:** The screening and binding affinity of the selected phytochemicals with RNAp were investigated through a molecular docking approach and the protein-ligand interactions were analyzed using suitable soft-wares. **Results:** The study reports that out of the nine selected phytochemicals of *N. sativa* 3 compounds (α-hederin, Dithymoquinone and Nigellidine) possessed significant docking scores/binding energies for RNAp of *M. tuberculosis*. The compound α-hederin ranked at the top in inhibiting the RNAp of *M. tuberculosis* as revealed by its lowest binding energy (-8.9 kcal moL^{-1}). **Conclusion:** Our results emphasize that α-hederin and dithymoquinone could be considered for ongoing drug development strategies against tuberculosis. However, further investigations are needed to confirm these findings.

Key words: *Nigella sativa*, tuberculosis, molecular docking, *in silico*, phytochemicals, drugs, RNA polymerase

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Corresponding Author: Shabir Ahmad Mir, Department of Medical Laboratory Sciences, College of Applied Medical Science, Majmaah University, Al Majmaah 11952, Saudi Arabia Tel: +966536300645

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

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). It has adversely affected human health since times of yore[1,2]. Globally, in 2019, an estimated 8.50 million TB cases and 1.18 million TB deaths have been reported among HIV-negative people[3]. The treatment of TB requires multi-drug regimens for at least 6 months. The 2 most important 1st-line drugs in the drug regimens are Rifampicin (RIF) and Isoniazid (INH). This conventional chemotherapy of TB exerts several adverse effects on patient health and its long duration is often linked with reduced patient compliance which can, in turn, lead to drug resistance. The emergence of Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) strains of MTB is continuously increasing and the patients with MDR- or XDR-TB require a prolonged course of multiple drugs which are more toxic, more expensive and not always effective[4]. The rise in drug-resistant TB over the previous decade is now a global public health emergency[5]. Although various anti-TB drug development efforts have been carried out by researchers across the globe in the recent past, the subsequent drug development pipeline which is currently available is still not adequate to address the global health challenge of TB. Therefore, there remains an urgent requirement to discover new anti-TB drugs affecting drug-susceptible as well as drug-resistant strains of MTB[5,6].

Natural products of plant origin and plant extracts have been used by humans as traditional remedies for tuberculosis management since ancient times. Therefore, one of the strategies to develop new anti-TB drugs is to test the promising phytochemicals from different plant sources[7]. In recent times, there has been improved interest in the investigation of natural sources for the identification of new anti-TB agents[8-12]. The wide screening of the phytochemicals in the laboratory is time-consuming as well as very expensive, but the process can be significantly hastened and made cost-effective by *in silico* screening through molecular docking approaches[6]. Among the various medicinal plants, *Nigella sativa* L. (Ranunculaceae) is one of the most precious nutrient-rich herbs with numerous medicinal benefits[12]. Several bioactive compounds from the seed of *N. sativa* (also called black cumin seeds) have been reported in the literature[13,14]. The *N. sativa* seeds are known for their traditional medicinal use in various ailments including airway disorders, diabetes, inflammation, infection, hypertension etc[12]. Moreover, a few recent studies have also reported the *in vitro* anti-tuberculosis efficacy of *N. sativa* seed extracts and some of its phytochemicals[15-17].

Keeping into consideration the various traditional medicinal uses of *N. sativa* seeds and their active components, this preliminary *in silico* study was designed to investigate the possible anti-TB potential of the main phytochemicals of *N. sativa*. To test our hypothesis, we applied a molecular docking approach to screening the selected *N. sativa* phytochemicals for their ability to bind and inhibit RNA polymerase (RNAp), a well-known pharmacological target of MTB.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Medical Laboratory Sciences, Majmaah University, Saudi Arabia from March-October, 2021.

Receptor/protein preparation for docking: The 3-dimensional (3D) crystal structure of *Mycobacterium tuberculosis* H37Rv RNAp (PDB ID: 5UHB) was obtained from the RCSB protein data bank (http://www.rscb.org) in PDB format. The protein structure was visualized and prepared for molecular docking using UCSF Chimera 1.12 software. Default settings of the software were used to prepare the protein structure that involved the removal of water molecules and the hetero-atoms, addition of polar hydrogens, the addition of appropriate charges and repair of missing atoms. The prepared structure was saved as a PDBQT file and used as input during the docking procedure.

Ligand/phytochemical preparation: The 3D structure of selected main phytochemicals of *N. sativa* and Rifampicin (a well-known anti-TB drug, used as positive control here) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and converted to PDB format using Pymol software. The PDB files of all the ligands were prepared for docking using UCSF Chimera 1.12 software. Ligand preparation was carried out by setting the torsion roots, the addition of gasteiger charges and assimilation of non-polar hydrogens. The prepared ligands were saved as PDBQT files and used as input during the docking procedure.

***In silico* molecular docking procedure:** A grid box covering the active site residues of the RNAp protein structure (PDB ID: 5UHB) was created by using AutoDock tools 1.5.7 and the grid parameters were saved and used as input during the docking procedure[18]. The molecular docking between the target protein and the individual ligands was performed using AutoDock Vina software[19]. The grid dimensions of RNAp (in Å) for active site-specific docking were searched from the

available literature and fixed at x: 20, y: 25, z: 30[20,21]. During the molecular docking through AutoDock Vina, the default exhaustiveness value of 8 and energy range of 4 was evenly fixed for all ligands. RIF was used as a positive control during the docking. AutoDock Vina results designate the docking scores as Gibbs free energy of binding (ΔG (kcal moL^{-1})) which in turn represents the efficacy of ligand binding to the chosen receptor[22]. Further, the different poses of the output files generated from docking experiments were converted to docking complexes (protein-ligand complexes) using PyMol molecular graphics system (https://pymol.org)[23] and the interaction of the ligands (in different poses) with the receptor (RNAp) were analyzed and visualized using BioVia Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download) and PyMol software[18].

Molecular dynamics studies: The Molecular Dynamics (MD) simulations of the docked complex of the top-ranked phytochemical was performed using the Molecular Dynamics on Web (MDWeb) program (https://mmb.irbbarcelona.org/MDWeb/). The root means square fluctuation (RMSF) of the selected complex was evaluated by using the CABS-flex server (http://212.87.3.12/CABSflex2/index).

RESULTS

Molecular docking of *N. sativa* phytochemicals: On screening the nine main phytochemicals of *N. sativa* through molecular docking analysis, we observed that 3 compounds including α-hederin (AHD), Dithymoquinone (DTQ) and Nigellidine (NGD) possess significant docking scores/binding energies (Table 1). These 3 compounds possess considerable binding affinity towards the active site of RNAp of MTB as indicated by their significantly lower binding energies on docking with RNAp. The structure, molecular weight and binding energies of all the 9 compounds screened in this study and the positive control RIF are given in Table 1. The compound AHD gives the lowest docking score/binding energy in complex with RNAp (ΔG = -8.9 kcal moL^{-1}) followed by DTQ (ΔG = -6.7 kcal moL^{-1}) and NGD (ΔG = -6.4 kcal moL^{-1}). Interestingly the binding energy/binding affinity of AHD was observed to be comparable to that of RIF (ΔG = -10.5 kcal moL^{-1}), a well-established inhibitor of RNAp and a prominent anti-tuberculosis drug, suggesting that AHD could be a possible inhibitor for RNAp of MTB.

Analysis and visualization of interactions in the docking complexes: The structure of RNAp (5UHB) and the binding of RIF and the top 3 phytochemicals (AHD, DTQ and NGD), in their best docking, poses to the active site of RNAp is shown in Fig. 1a-d. Keeping in consideration the 2 residues (His 451 and Ser 456) which are very important for binding the standard anti-TB drug, RIF to the binding pocket of the RNAp, we fixed the grid dimensions around these residues and docked the phytochemicals accordingly. After visualizing the docking complexes, we observe that the phytochemicals bind

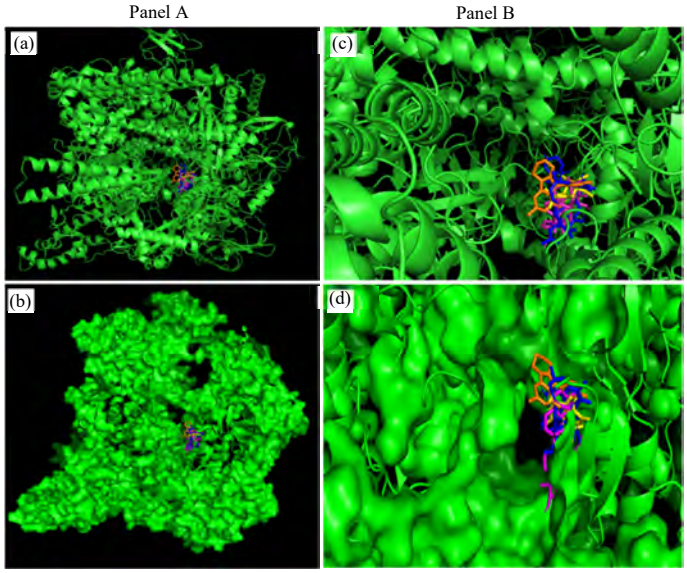
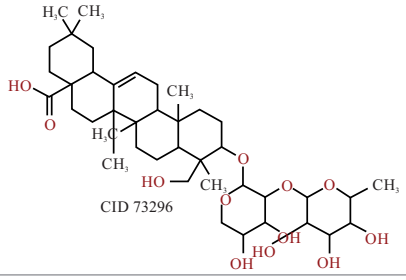
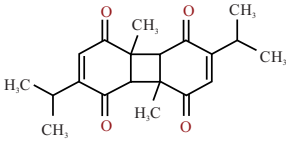
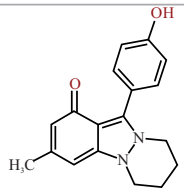
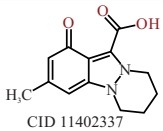
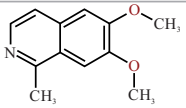
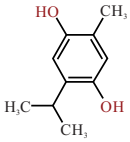
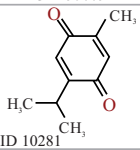
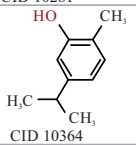
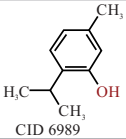
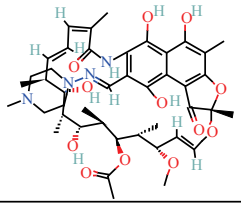


Fig. 1(a-d): Demonstration of the binding of the top-ranked phytochemicals of *N. sativa* and the control drug to the active site of RNAp of MTB, Panel A: (a) Cartoon and (b) Surface representation of RNAp with the ligands bound to its active site, Panel B: (c) Magnified cartoon and (d) Surface view of the active site of RNAp occupying the ligands
RNAp is shown in green, whereas the ligands AHD, DTQ, NGD and RIF are shown in magenta, yellow, orange and blue colour, respectively

Table 1: Chemical structure of the selected *Nigella sativa* phytochemicals and their respective docking scores against RNA polymerase of *Mycobacterium tuberculosis*

Phytochemicals/ligands	Chemical structure	Molecular weight (g moL^{-1})	Docking score (kcal moL^{-1})
α- Hederin	 CID 73296	750.97	-8.9
Dithymoquinone	 CID 398941	328.41	-6.7
Nigellidine	 CID 136828302	294.35	-6.4
Nigellicine	 CID 11402337	246.27	-6.0
Nigellimine	 CID 20725	203.24	-5.5
Thymohydroquinone	 CID 95779	166.22	-4.6
Thymoquinone	 CID 10281	164.20	-4.7
Carvacrol	 CID 10364	150.22	-5.0
Thymol	 CID 6989	150.22	-4.6
RIFAMPICIN (control drug)		822.9	-10.5

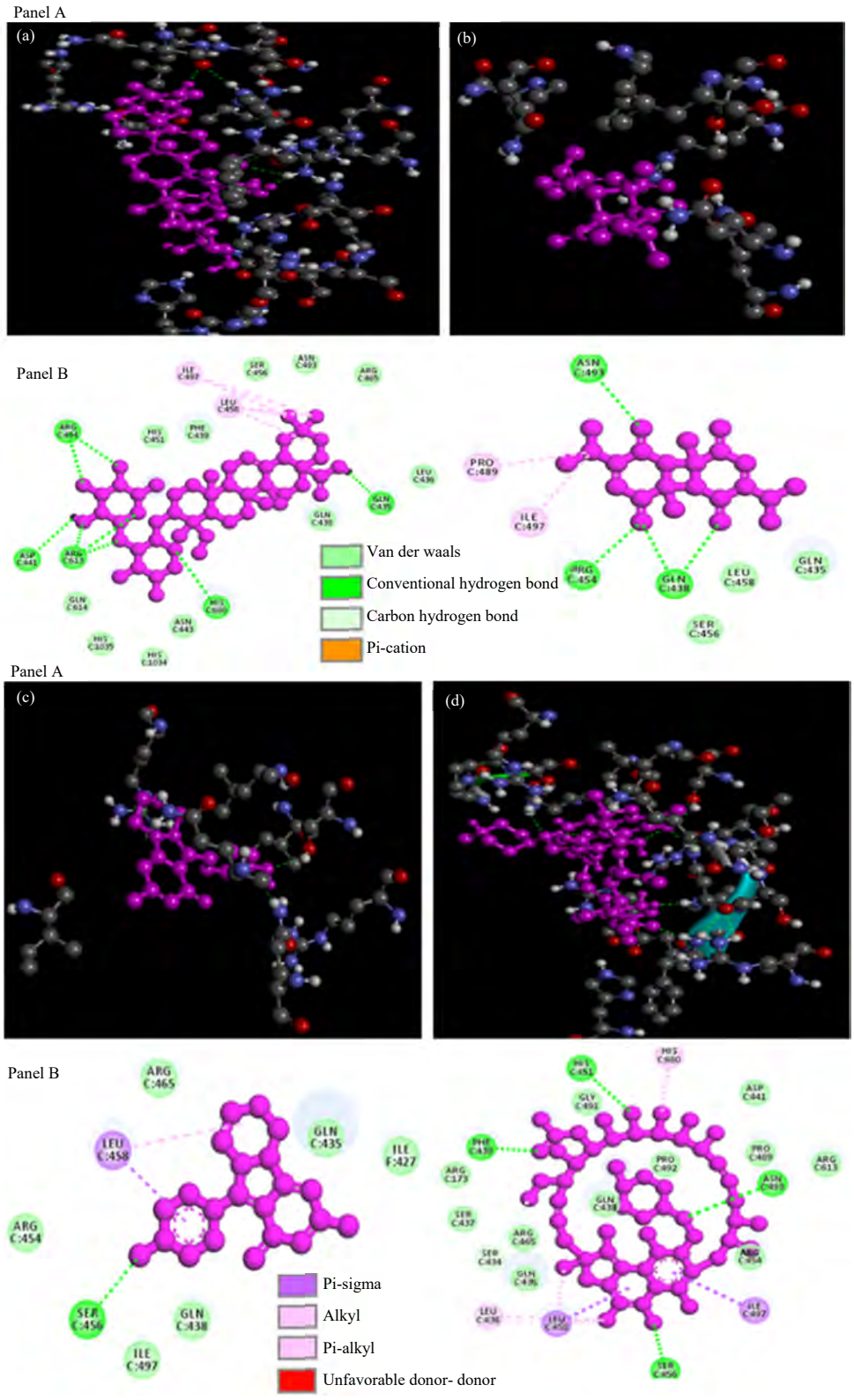


Fig. 2(a-d): Molecular docking analysis of the 3 top-ranked compounds of *N. Sativa* (a) AHD, (b) DTQ, (c) NGD and (d) control drug RIF upon docking with RNAp of MTB

Panel A and B show the respective 3 and 2 dimensional illustrations of the interaction of ligands with specific amino acid residues in the active site of RNAp. All the ligands (AHD, DTQ, NGD and RIF) are shown in magenta colour

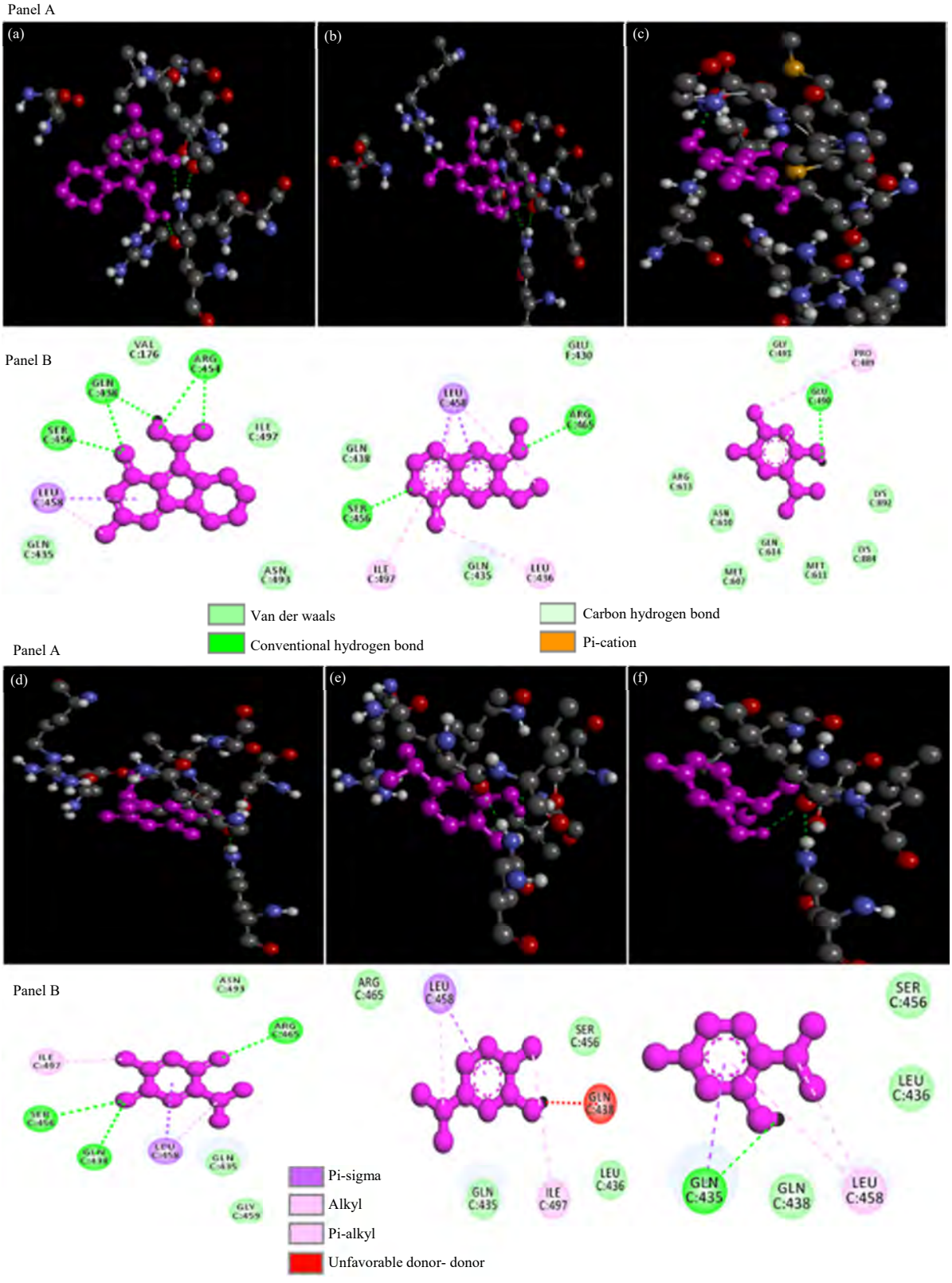


Fig. 3(a-f): Molecular docking analysis revealing the binding positions of the other six phytochemicals of *N. sativa*, (a) Nigellicine, (b) Nigellimine, (c) Thymohydroquinone, (d) Thymoquinone, (e) Carvacrol and (f) Thymol

Panel A and B show the respective 3 and 2 dimensional diagrams displaying the ligand interactions with specific amino acid residues in the active site of RNAp

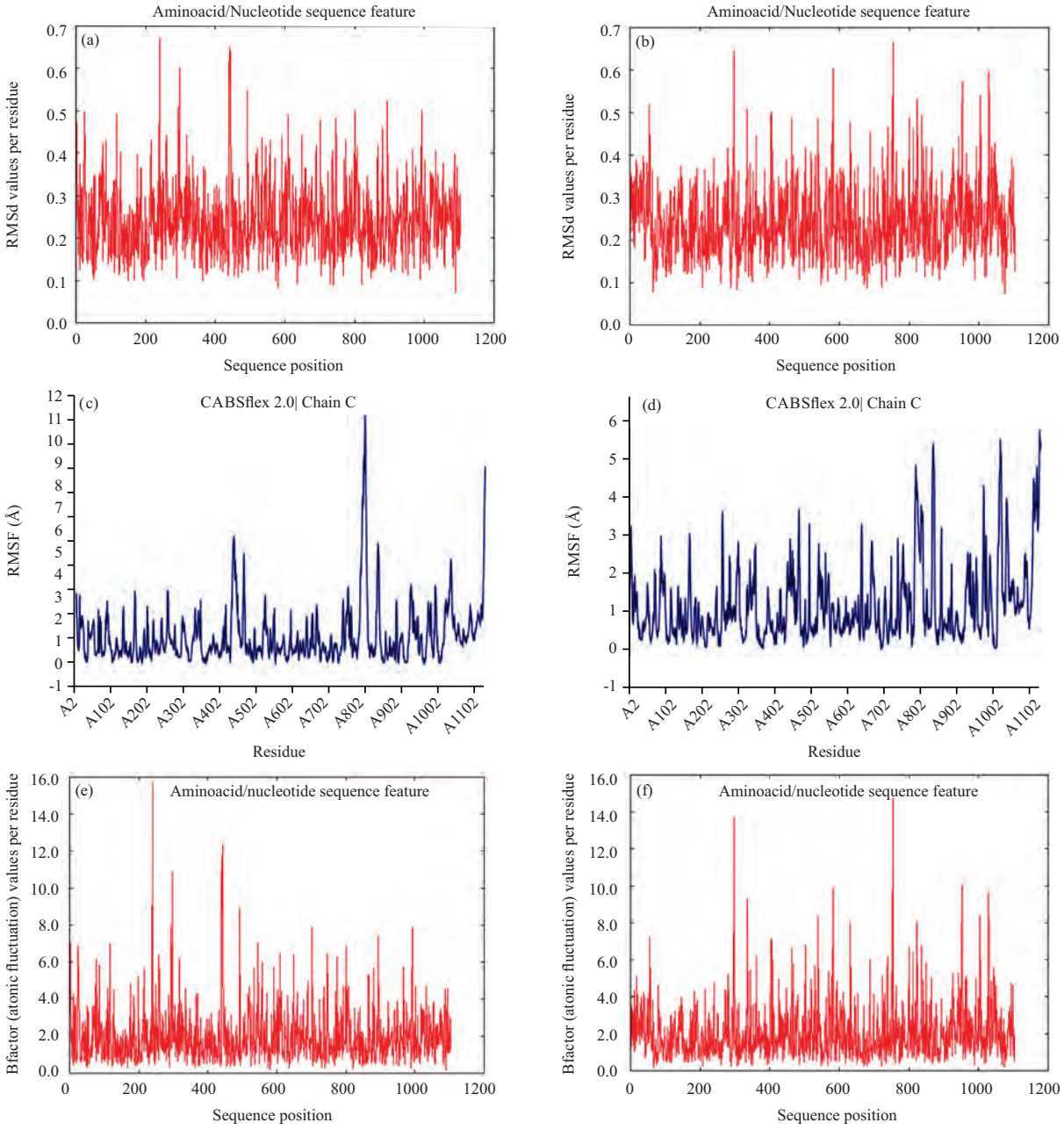


Fig. 4(a-f): Molecular dynamic simulations of the RNAp/α-hederin docking complex,(a) Root mean square deviation (RMSD) profile of the RNAp chain C alone, (b) RMSD profile of the RNAp chain C/α-hederin complex, (c) Root mean square fluctuations (RMSF) profile of the RNAp chain C alone, (d) RMSF profile of the RNAp chain C/α-hederin complex, (e) Atomic fluctuations of the RNAp chain C alone and (f) Atomic fluctuations of the RNAp chain C/α-hederin complex

exactly in the binding pocket of RNAp as RIF does (Fig. 1a-d). Following the visualization of all the poses of the ligands in the corresponding docking complexes by PyMol, the docking complexes with the best ligand poses were selected and analyzed for the interactions between the specific ligand and the active site residues of RNAp. The 2 Dimensional (2D) and 3D visualization of the interaction of RIF and the top-ranked phytochemicals (AHD, DTQ and NGD) with the active site residues of RNAp is shown in Fig. 2a-d, whereas, the interaction of RNAp with the rest of the 6 phytochemicals is shown in (Fig. 3a-f).

The top-ranked phytochemicals, AHD, DTQ and NGD interacted with the active site residues of RNAp through 8, 4 and 1 conventional hydrogen bonds, respectively. In addition

to the hydrogen bonds, there are also some non-bonded interactions (like van der Waals forces, pi-alkyl, pi-cation etc.) between these phytochemicals and the active site residues of RNAp as depicted in the 2D illustration (Fig. 2). The interaction of various active site residues of RNAp with RIF is shown in Fig. 2. Based on the lowest binding energy and efficient interaction of AHD with the active site residues of RNAp, we here report AHD as a lead molecule that has the potential to inhibit the RNAp of MTB and is worth investigating further for confirming its inhibitory potential. For further confirmation, the top-ranked phytochemicals (AHD, DTQ and NGD) were docked with a single chain of RNAp (Chain C, the core enzyme) and it was observed that AHD, DTQ and NGD bind into the active site of the chain C of RNAp with a docking score of -8.7, -6.2 and -6.0 kcal moL^{-1}, respectively. Moreover, the stability of the docking complex of the top-ranked phytochemical, α-hederin with chain C of RNAp (RNAp/α-hederin docking complex) was confirmed by molecular dynamic simulation and the results are shown in (Fig. 4a-f).

DISCUSSION

In this study, RNAp of MTB was used as the target enzyme for screening the anti-TB potential of the selected phytochemical compounds of *N. sativa* using a molecular docking approach. The rationale for the selection of this particular protein as a target enzyme was that it is indispensably involved in RNA synthesis in MTB and is, therefore, a key enzyme required for its growth and survival within the eukaryotic host. RNAp is one of the well-known and important drug targets of MTB and is inhibited by one of the clinically potent anti-TB drugs, namely, RIF. However, due to the emergence of drug resistance to anti-TB drugs in general and RIF in particular, there is a critical need to develop alternative drugs which could replace this drug or other drugs in anti-tuberculosis chemotherapy. Therefore, we undertook this *in silico* study as a preliminary attempt to discover new anti-TB drugs from natural phytochemicals of *N. sativa* using a molecular docking approach. Molecular docking is a standard procedure used in the virtual screening of various ligands against protein targets and several studies have investigated the interaction of natural products against specific mycobacterial enzymes using this approach[4,6,24-27]. *N. sativa* is well recognized for its anti-microbial, anti-inflammatory and immunostimulatory activities and its beneficial effects and safety in different diseases is well established in the literature[28-36]. The *in silico* screening of phytochemicals of *N. Sativa* against various drug targets of SARS-Cov-2 has been reported in several studies[14,37-41], however, there are only a few studies that highlight the anti-mycobacterial potential of *N. Sativa*[15-17]. On applying the *in silico* screening strategy, we observed that out of the 9 screened phytochemicals of *N. sativa*, 3 (AHD, DTQ and NGD) exhibited the potential to inhibit RNAp as indicated by their low binding energies (Table 1). Among these AHD possessed the lowest binding energy (ΔG = -8.9 Kcal mol^{-1}) and hence the highest affinity for RNAp. Its binding energy was slightly lower but comparable to that of the standard anti-TB drug, RIF (ΔG = -10.5 kcal moL^{-1}). Therefore, AHD can be used as a potential lead compound for anti-TB drug discovery.

In this study, we used the X-Ray crystal structure of RNAp (PDB ID: 5UHB) for screening the phytochemicals using RIF as a controlled drug for comparison. In *M. tuberculosis* $H_{37}Rv$ there are 2 important residues (His451 and Ser456) in the binding pocket of RNAp which are very important for binding of RIF to RNAp and thus for its inhibition. The change in any of these 2 residues through the mutation in the respective codons leads to RIF resistance in MTB[42,43]. Therefore, we fixed the grid dimensions around these 2 residues to perform rigid docking of the phytochemicals at the desired location of RNAp. After visualizing the RNAp-phytochemical and RNAp-standard drug (RIF) docking complexes using PyMol, it was observed that the selected phytochemicals bind within the same binding pocket in the target protein where RIF does (Fig. 1). The top 3 compounds (AHD, DTQ and NGD), especially AHD efficiently bind to the binding pocket of RNAp and stably interact with one or more interacting residues present in its active site (Fig. 2). The interaction involves strong bonds (including hydrogen bonds, pi cation, pi anion bonds etc.) and might lead to the inhibition of the activity of this enzyme. Interestingly it was observed that the 2 top-ranked compounds AHD and DTQ showed interaction with various residues other than His451 and Ser456 in the binding site of RNAp (Fig. 2). These unique interactions of AHD and DTQ in the binding site of RNAp indicate that these compounds might inhibit the RNAp of RIF-resistant strains of MTB as well. Based on the findings of our study, we suggest that *N. Sativa* phytochemicals especially AHD and DTQ are worth studying further through *in vitro* biological evaluation.

CONCLUSION

In the present study, a molecular docking approach was used to identify potential anti-TB compounds from the major phytochemicals of *N. sativa*. Based on their docking score and stable interactions with the RNAp of MTB, we identified 3 phytochemicals (AHD, DTQ and NGD) from *N. sativa* that might inhibit MTB. Our results show that these top 3 potential

phytochemical compounds of *N. sativa*, especially AHD and DTQ could be considered for ongoing drug development strategies against drug-susceptible as well as drug-resistant TB. However, further biological studies are required to confirm the findings of this preliminary *in silico* study. Our findings support the existing research evidence on the traditional use of *N. sativa* or its extracts for the treatment of tuberculosis.

SIGNIFICANCE STATEMENT

This study discovers the possible RNAp inhibitory potential of the phytochemicals of *N. sativa* that can be beneficial for the management of tuberculosis. Due to the various limitations of currently available anti-TB drugs, the discovery of new effective drugs for tuberculosis is essentially needed and various trials are already being carried out by researchers across the world to meet this demand. Therefore, this study adds up significant information to the available literature and the top-ranked phytochemicals reported here could be useful for designing drug development strategies against TB. Interestingly, this study also explains the possibility of these compounds being effective against drug-resistant tuberculosis.

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