Research Article

Molecular Modeling and Designing of Inhibitors against DevR (P9WMF8) Protein of *Mycobacterium tuberculosis*

Mahendran Radha*, Annie Absala Trace, Jeyabaskar Suganya, Agnal Vincent Paul
Department of Bioinformatics, School of Life Sciences, Vels University, Pallavaram, Chennai, Tamil Nadu, India.
***Corresponding author's E-mail:** mahenradha@gmail.com

Accepted on: 24-09-2015; **Finalized on:** 31-10-2015.

ABSTRACT

Tuberculosis is a main health hazard throughout the world due to its multidrug resistance. The DevR-DosR belongs to two component regulatory system and they were reported to be more concerned in dormancy response of *Mycobacterium tuberculosis*. In the current study three dimensional structure of transcriptional regulatory protein DevR (Uniprot ID: P9WMF8) was predicted using modeler 9v 11. Few natural antituberculosis compounds from plants and few synthetic compounds which possess potential activity against multi drug resistant tuberculosis were selected by performing a thorough literature Search. Both Natural and Synthetic compounds were analyzed for drug likeliness based on the Lipinski's rule of Five. Finally the compounds which strictly adhere to Lipinski's rule were selected and Molecular docking was performed against DevR (P9WMF8) using Argus lab. Finally after the comparison of natural and Synthetic compounds, the natural compounds were predicted to have high binding interaction with DevR. This study serves as a potential insight to identify new drugs against Multi drug resistant tuberculosis.

Keywords: DevR, *Mycobacterium tuberculosis*, Homology modeling, Docking, Natural Compounds, P9WMF8

INTRODUCTION

Mycobacterium tuberculosis is a pathogenic bacterium which is the causative agent of the deadly disease tuberculosis. On a recent survey, it was reported that nearly every year the ratio of death cases was increased from 2 million to 9 million.[1] Millions of people of all age category died from tuberculosis every year.[2] The disease acquired resistance to most of the existing antibiotics, and is reported to be multi-drug resistant (MDR).[3] *Mycobacterium tuberculosis* is mostly transmitted throughout the respiratory route and causes active tuberculosis in unhygienic persons. The host reaction to the organism is a major determinant to the conclusion of infection, and cell-mediated immunity plays an essential role in host resistance for this tedious disease.[4] *Mycobacterium tuberculosis* spread during aerosols and causes pulmonary and extra pulmonary tuberculosis where it infects human lungs and other body parts. In infected body parts, bacilli stay dormant for a longer time and get reactivated in immunosuppressed conditions.[5] It is observed that majority of difficulties are faced during the treatment of MDR tuberculosis, many tuberculosis patients are likely to be infected with human immunodeficiency virus (HIV).[6] Tuberculosis is the most general opportunistic infection (OI) among HIV-infected individuals.[7]

The two-component regulatory system DevR/DevS (DosR/DosS) involved in onset of the dormancy response. This two component systems play an essential role in the variation of pathogenic bacteria in the atmosphere prevailing within the host tissues. The gene DevR is responsible for regulation of encoding.[8] The DevR was recognized among genes differently expressed in the virulent strain of *Mycobacterium tuberculosis*. The drug likeness of compounds can be predicted by the Lipinski's rule of five. The molecular docking process predicts ligand verification and orientation within their targeted binding site. *In silico* docking studies of the bioactive compounds against DevR protein was found to be very useful in identification of potent inhibitors against tuberculosis.

As millions of drugs are accessible for healing of *Mycobacterium tuberculosis,* the clinical development of these drugs has revealed increased amount of side effects. This has been the foundation for the development of anti tuberculosis drug which includes herbal drugs.[9] Ciprofloxacin, Levofloxacin, ofloxacin, and sparfloxacin are the synthetic drugs which are showing activity against some multi-resistant bacteria.[10] The natural drugs like Quercetin, Lignans, Xanthone, Coumarin, Piperine are phytoconstituents that present in plant extract and they possess good pharmacological effect, and act as natural anti-tubercular agents.[11] In the present study, we have analyzed the drug–likeness of Natural and synthetic compounds, using Lipinski's rule of five and investigated the binding mechanism of compounds with transcriptional regulator protein DevR.

MATERIALS AND METHODS

Target sequence and template identification

The target sequence was retrieved from Uniprot Id: **P9WMF8** and this target sequence was used as an input in pdbsum for identifying template. For modeling, a protein template was identified (3c3w).

Homology modeling of protein DevR (P9WMF8)

To identify the structural and functional information of Transcriptional regulatory protein DevR (DosR) the 3D structure is considered to be an important parameter.

The experimental structure of Transcriptional regulatory protein DevR (**P9WMF8**) is unavailable in structural databases protein data bank; hence, Transcriptional regulatory protein DevR structure was predicted using an homology modeling method using Modeller 9v11 and the predicted structure is analyzed using Ramachandran plot. The homology modeling requires a template sequence of known three dimensional (3D) structures and the template identified for the particular structure study was 3c3w.

Active site identification of DevR

CASTP - server identified 20 active pockets for the protein DevR. The identification is based on specific computational geometry process.[12]

Procheck

The modeled DevR Protein was validated using procheck. The quality of DevR protein was checked and analyzed by using Ramachandran Plot via Rampage server.

Lipinski's rule of five

The drug likeness values was calculated based on log P, molecular weight, number of hydrogen donors, number of hydrogen acceptors and Molar refractivity. Based on these properties the compounds which adhere to Lipinski's rule were selected for the study.

Identification of ligands

Natural antituberculosis compounds used for tuberculosis treatment were identified and retrieved from Pubmed Literature. Quercetin, Lignans, Coumarin, xanthone and Piperine are natural plant compounds used in the treatment of tuberculosis.[13] Synthetic compounds which show potential activity against Multidrug-resistant tuberculosis are Ciprofloxacin, Rifampicin, levofloxacin, Ofloxacin, Ethambutol, Isoniazid and Sparfloxacin drugs.[14,15] Natural and the Synthetic compounds which possess anti-tuberculosis activity and which passed in Lipinski's rule of five was used for further docking study. They are Ciprofloxacin, levofloxacin, ofloxacin, and sparfloxacin, Quercetin, Xanthone, Piperine. These compounds were optimized using Chemsketch software. Using draw mode of Chemsketch, the ligands were generated and the 3D optimizations were prepared and was saved in mol file format. Binding interaction of the ligands where performed according to the calculation method by Argus Lab 4.0.1 software.

Docking studies using Argus Lab 4.0.1

Molecular interaction studies were performed using Argus lab software using bioactive compounds.[16] The interaction of natural compound with target protein is important in the drug development process. The possible binding sites of Protein DevR were searched using CASTP. All the compounds were docked into the active site of DevR receptor protein. After completion of docking, the docked protein (protein-ligand complex) was analyzed. Further the docking poses were saved for each compound and they were ranked according to their dock score function.

RESULTS AND DISCUSSION

Construction of 3-D models

The sequence of Transcriptional regulatory protein DevR was retrieved from the Uniprot sequence database (www.uniprot.org) with the accession number P9WMF8. The 3D structure of Transcriptional regulatory protein DevR was predicted by homology modeling method, and the known experimental template protein structure was selected based on the identity (sequence identity is 100%). The Crystal structure of the *Mycobacterium Tuberculosis* hypoxic regulator 3c3w A chain was taken as a template structure with the resolution of 2.20 Å from PDB database (PDB ID: 3c3w A chain). Sequence alignment was performed for the Crystal structure of the *Mycobacterium tuberculosis* hypoxic regulator protein with the target protein using ClustalW. **(Fig.1)** The selected template and target protein sequences were aligned perfectly. Based on the alignment, the initial 3D protein model was predicted using Modeller 9 v11. (**Fig.2)**

```
CLUSTAL W (1.83) multiple sequence alignment


3c3w_A                  MVKVFLVDDHEVVRRGLVDLLGADPELDVVGEAGSVAEAMARVPAARPDVAVLDVRLPDG
sp|P9WMF8|DEVR_MYCTO    MVKVFLVDDHEVVRRGLVDLLGADPELDVVGEAGSVAEAMARVPAARPDVAVLDVRLPDG
                        ************************************************************

3c3w_A                  NGIELCRDLLSRMPDLRCLILTSYTSDEAMLDAILAGASGYVVKDIKGMELARAVKDVGA
sp|P9WMF8|DEVR_MYCTO    NGIELCRDLLSRMPDLRCLILTSYTSDEAMLDAILAGASGYVVKDIKGMELARAVKDVGA
                        ************************************************************

3c3w_A                  GRSLLDNRAAAALMAKLRGAAEKQDPLSGLTDQERTLLGLLSEGLTNKQIADRMFLAEKT
sp|P9WMF8|DEVR_MYCTO    GRSLLDNRAAAALMAKLRGAAEKQDPLSGLTDQERTLLGLLSEGLTNKQIADRMFLAEKT
                        ************************************************************

3c3w_A                  VKNYVSRLLAKLGMERRTQAAVFATELKRSR------
sp|P9WMF8|DEVR_MYCTO    VKNYVSRLLAKLGMERRTQAAVFATELKRSRPPGDGP
                        ******************************
```

Figure 1: Sequence alignment of DevR protein with the template structure 3c3w depicts the conserved region in stars (*) and deleted regions with dashes (-)

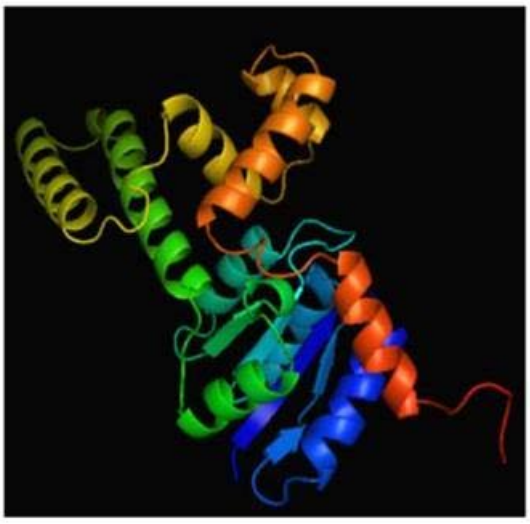

Figure 2: 3D Model of Transcriptional regulatory protein DevR (DosR)

The Secondary structure prediction of Protein DevR contain 58.06% of alpha helix and 11.08% of beta turn and 17.05 % of random coil.

The Modeled 3D structure was validated using Ramachandran plot. Ramachandran plot shows 99.5% allowed region. In the predicted model, 99.5% of the residues were in the most favored region, 0.5% in allowed region, 0% of the residues lying in the disallowed regions. The above results clearly indicate the quality of predicted protein structure is perfect. (**Fig.3)**

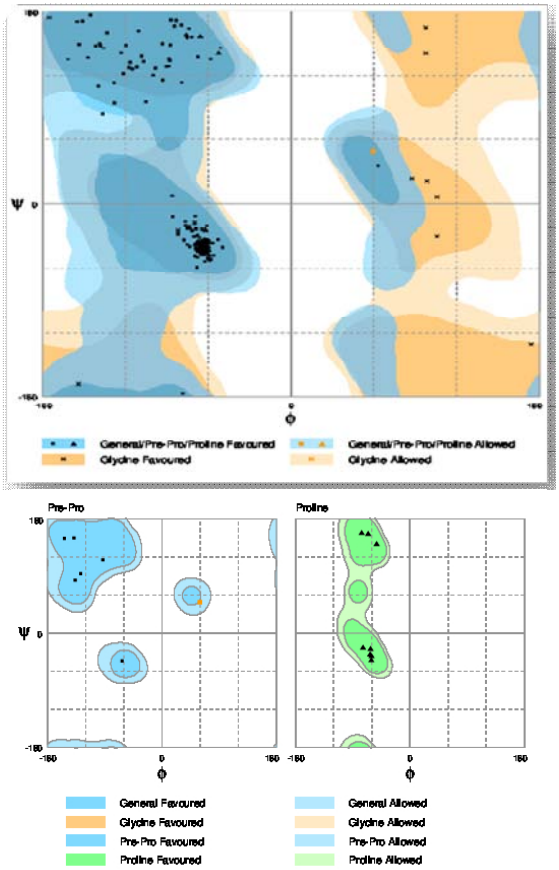

Figure 3: RAMPAGE: Assessment of the Ramachandran Plot

By validating the DevR protein model in Ramachandran plot 99.5% of the residues were present in the most favored region, 0.5% in allowed region, 0% of the residues in the disallowed regions.

Active site of the protein

The exposure of ligand-binding sites is frequently the initial point for protein function detection and drug discovery. 3D ligand site server predicts the active site of the protein DevR. The active site of predicted DevR model comprises of amino acid residues such as MET109, ALA112,ARG113,VAL115,LYS116,ARG138,LEU147,ASP152, ARG155,THR156,LEU158,GLY159.

Lipinski's rule of five

The natural plant compounds *Xanthonefrom Canscora decussate*, Quercetin from *Myrtuscommunis* and Piperine from *Piper species*[17-19] and Synthetic compounds like Ofloxacin, Ciprofloxacin, Levofloxacin, Sparfloxacin were passed in Lipinski's rule which consists of following parameters a. Molecular mass less than 500 Dalton, b. logP less than 5, c. Hydrogen bond donors Less than 5, d. Hydrogen bond acceptors Less than 10, e. Molar refractivity should be between 40-130 **(Table.1)**.

Docking

To know the interactions between the ligands and DevR protein and to investigate their binding mode, Molecular docking study was performed using Argus dock accessible under Argus Lab 4.0.1.[20]

Docking scores: Interaction Profiles

The docking result of selected Natural and Synthetic Compounds with DevR protein in 3 dimensional views are shown in **(Fig.4 & Fig.5).** Red color represents binding site region of residues; violet color represents the ligand; blue, yellow and green color represents the rest of the protein. The following docking scores were obtained. Ofloxacin with docking score of -6.31 Kcal/mol, Levofloxacin with docking score of -6.30 Kcal/mol, Sparfloxacin with docking score of -6.77 Kcal/mol respectively, Natural drug compounds Xanthone with docking score -7.18kcal/mol, Quercetin with docking score -6.4215kcal/mol, and the docking score was maximum for Piperine with –7.54 kcal/mol. The above natural and synthetic compounds bind to the active site region of the DevR protein and their hydrogen bond interaction profiles are shown in (**Table.2 and Table.3**).

Ciprofloxin with binding energy of -7.1 Kcal/mol, the amino acid 160LEU binds with atom 1175N with distance 2.5 and amino acid 159GLY binds with atom 1171N with distance of 2.9 are showed in Fig4(a). Levofloxacin with binding energy of -6.30 Kcal/mol, the amino acid 155 ARG binds with atom 1142N with distance 2.3 are showed in Fig4(b). Sparfloxacin with binding energy of -6.77 Kcal/mol, the amino acid 160LEU binds with atom 1175N with distance of 2.8, amino acid 159GLY binds with atom 1171N with distance of 2.5 are showed in Fig4(c). Ofloxacin with binding energy of -6.31 Kcal/mol, the amino acid 115ARG binds with Atom 114N with the distance of 2.9, the amino acid 138ARG binds with atom 1016N with the distance of 2.8 and amino acid 138ARG binds with atom 1019N distance of 2.6 are showed in Fig4 (d). Quercetin with binding energy of -6.42Kcal/mol, the amino acid 155ARG binds with atom 1145N with distance of 2.8 are showed in Fig5(e). Piperine with binding energy of -7.54 Kcal/mol, the amino acid 156THR binds with atom 1148N with distance of 2.9, amino acid 159GLY binds with atom 1171N with distance of 2.9 are showed in Fig5(f). Xanthone with binding energy of -7.18 Kcal/mol, the amino acid 156THR binds with atom 1148N with distance of 2.9 are showed in Fig5(g).

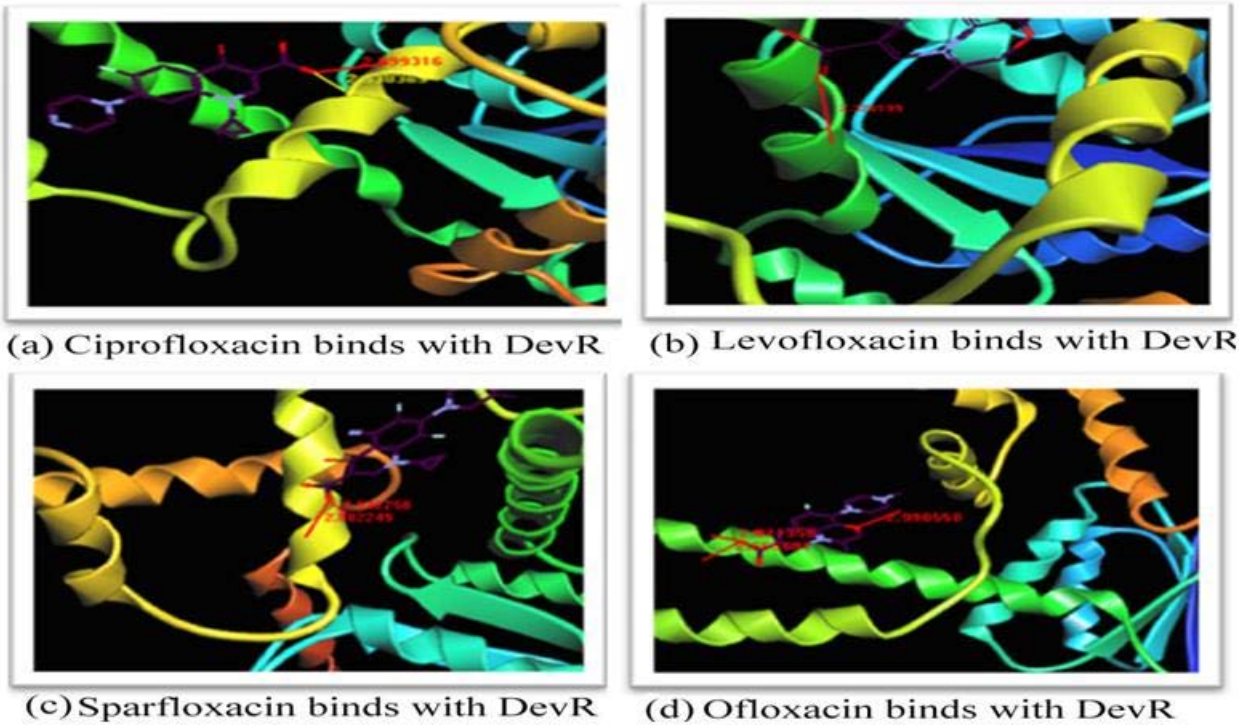

Figure 4: Docking complex of Transcriptional regulatory protein DevR (DosR) with Synthetic Compounds

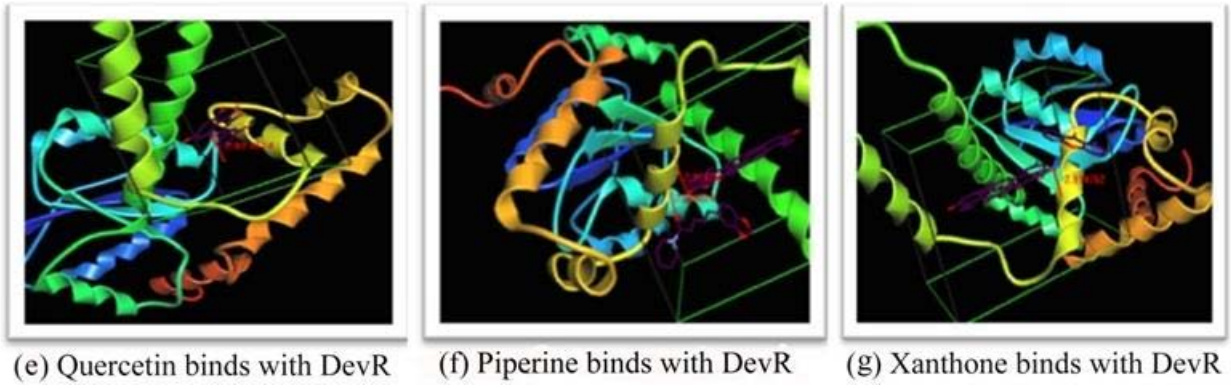

Figure 5: Docking complex of Transcriptional regulatory protein DevR (DosR) with Natural Compounds

Table 1: Lipinski's properties of the compound

S. No	Molecule	Mass<500	Log P<5	H-Donar<5	H Acceptor<10	Molar Refractivity 40-130
1	Rifampicin	800.0	0.1	2	13	193.2
2	Isoniazid	137.0	-0.3	3	4	35.8
3	Levofloxacin	192.0	1.9	2	4	56.0
4	Ciprofloxacin	330.0	-0.6	1	5	81.9
5	Ethambutol	714.0	-1.5	1	12	166.8
6	Ofloxacin	356.0	-0.1	1	5	88.8
7	Sparfloxacin	480.0	-1.9	4	1	128.9
8	Piperine	285	3.1	0	4	79.7
9	Quercetin	304	1.8	3	7	74.5
10	Xanthone	312.0	0.1	4	6	77.1
11	Coumarin	146	0	2	1.6	31.1
12	Lignans	1362	13	32	7.7	323.4

Table 2: Binding energy of Synthetic Compounds with Transcriptional Regulatory Protein DevR

S. No	Synthetic Compounds Name	Binding Energy	Hydrogen Bond Interaction Amino acid - Atom
1	Ciprofloxin	-7.16kcal/mol	DISTANCE 2.530369 (160LEU -1175N) 2.999316 (159GLY-1171N)
2	Levofloxacin	-6.30kcal/mol	DISTANCE 2.320199 (155ARG -1142N)
3	Sparfloxacin	-6.77kcal/mol	DISTANCE 2.882249 (160LEU -1175N) 2.542258 (159GLY-1171N)
4	Ofloxacin	-6.31kcal/mol	DISTANCE 2.998558 (115ARG -114N) 2.071358 (138ARG-1016N) 2.665687 (138ARG-1019N)

Table 3: Binding energy of Natural Compounds with Transcriptional Regulatory Protein DevR

S. No	Natural Compounds Name	Binding Energy	Hydrogen Bond Interaction Amino acid - Atom
1	Quercetin	-6.4215kcal/mol	DISTANCE 2.823074 (155ARG-1145N)
2	Piperine	-7.54kcal/mol	DISTANCE 2.9147262 (156THR -1148N) 2.998224 (159GLY-1171N)
3	Xanthone	-7.18kcal/mol	DISTANCE 2.914762 (156THR-1148NN)

CONCLUSION

Tuberculosis remains a leading infectious killer disease world-wide and hence there is an urgent need for the development of a new anti tuberculosis drug. In this work, we performed various approaches like molecular modeling of Transcriptional regulatory protein DevR, molecular interaction studies between target and ligand which helped us to find the suitable anti tuberculosis inhibitors. Molecular docking continues to embrace great guarantee in the field of computer based drug design which screens small bioactive molecules by orienting and scoring them in the binding site of a protein. Synthetic compounds like Ciprofloxacin, Levofloxacin, Ofloxacin, and Sparfloxacin interacted with following active site of DevR protein with high binding affinity (ARG155, GLY159 and ARG138). Natural Plant compounds Piperine, Quercetin, Xanthone interacted with active site region 156THR, 159GLY, 155ARG. While Comparing the docking analysis of both synthetic and natural compounds, both the compounds bound well in the active site of DevR. But the natural compounds showed the highest binding affinity with a value of -7.54kcal/mol. Several studies reported that plant drugs show fewer side effects than synthetic compounds. *In silico* drug designing studies reduces time and energy to a smaller extent and it is affordable. The outcome of docking result revealed that the target protein exhibits the good binding energy towards the natural compounds and further clinical trials can be performed for the above natural compounds

which can act as good inhibitors against *Mycobacterium tuberculosis*. Therefore, this approach is important for drug discovery process and therapy of tuberculosis.

REFERENCES

1. Matteelli A, Carvalho AC, Dooley KE, Kritski A, TMC207 : the first compound of a new class of potent antituberculosis drugs, Future Microbial, 5(6), 2010, 849-858.
2. Dubey D, Rath S, Sahu CM, Debata KN, Padhy NR, Antimicrobials of plant orgin against TB and other infections and Economics of plant drug- Introspection, Indian journal of Traditional Knowledge, 11(2), 2012, 225-233.
3. Sharma A, Dutta P, Sharma M, Rajput KN, Dodiya B, Georrge JJ, Kholia T, Consortium OSDD, Bhardwaj A, BioPhytmol:a drug discovery community resource on anti-mycobacterial phytomolecules and plant extracts, Journal of Cheminformatis, 6(46), 2014, 1-10.
4. Bhattacharya D, Dwivedi PV, Maiga M, Maiga M, Kaer VL, Bishai RW, Das G, Small Molecule-directed Immunotherapy against Recurrent Infection by Mycobacterium tuberculosis, The journal of Biological Chemistry, 289(23), 2014, 16508-16515.
5. Santhosh SR, Suriyanarayanan B, Plant:A source for New Antimycobacterial Drugs, Planta Med, 80(1), 2014, 9-12.
6. Lin HH, Dowdy D, Dye C, Murrayd M, Cohen T, The Impact of New Tuberculosis diagnostics on transmission: why context matter, Bull world health organ, 90(10), 2012, 739-747.
7. Padmapriyadarsini C, Narendran G, Swaminathan S, Diagnosis & treatment of tuberculosis in HIV co-infected patients, Indian Journal of Medical Research, 134(6), 2011, 850-865.
8. Saini DK, Malhotra V, Dey D, Pant N, Das TK, Tyagi JS, Dev R-Dev S is a bona fide two component system of Mycobacterium tuberculosis that is hypoxia-responsive in the absence of the Dna-Binding domain of DevR, Microbiology, 150(4), 2004, 865-875.
9. Ittiyavirah PS, Paul M, *In silico* docking analysis of constituents of Zingiber Officinale as anti depressant, Journal of Pharmacognosy and Phythoteraphy, 5(6), 2013, 101-105.
10. Al-abdeen ZSS, Abdullah I, The synergism effect of aqueous garlic extract and ciprofloxacin against some multi-resistant bacteria, Journal of Microbiology and Biotechnology Research, 3(3), 2013, 136-142.
11. Arya V, A Review on Anti-Tubercular Plants, International Journal of PharmTech Research, 3(2), 2011, 872-880.
12. Singh S, Gupta AK, Verma A, Molecular Properties and Bioactivity score of the Aloe vera antioxidant compounds – in order to lead finding, Research Journal of Pharmaceutical, Biological and Chemical Science, 4(2), 2013, 876.
13. Narad P, Jain A, Sengupta A, Docking Studies For Tuberculosis Taking Alanine Racemase As A Receptor And A Novel Plant Source Quercetin As A Potential Drug Source, International Journal of Pharma Bio Sciences, 5(3), 2014, 31–39.
14. Gupta R, Thakur B, Singh P, Singh BH, Sharma DV, Katoch MV, Chauhan SVS, Anti-tuberculosis activity of Selected medicinal Plant against multi drug resistant Mycobacterium tuberculosis isolates, Indian Journal of Medical Research, 2010, 809-813.
15. Richeldi L, Covi M, Ferrara G, Franco F, Vailati P, Meschiari E, Fabbri LM, Velluti G, Clinical use of Levofloxacin in the long-term treatment of drug resistant tuberculosis, Monaldi Arch Chest Dis, 57(1), 2002, 39-43.
16. Naz A, Bano K, Bano F, Ghafoor NA, Akhtar N, Conformational Analysis (Geometry Optimization) Of Nucleosidic Antitumor Antibiotic Showdomycin By Argus lab 4 Software, Pakistan Journal of Pharmaceutical Sciences, 22(1), 2009, 78-82.
17. Awoyinka OA, Oyewole OI, Comparative pesticidal activity of dichloromethane extracts of Piper nigrum against Sitophiluszeamais and Callosobruchus maculates, African Journal of Biotechnology, 5(24), 2006, 2446-2449.
18. Małgorzata M, Quercetin And Its Derivatives: Chemical Structure And Bioactivity – A Review MałgorzataMaterska, Polish Journal Of Food And Nutrition Sciences, 58(4), 2008, 407-413.
19. Negi JS, Bisht VK, Naturally Occurring Xanthones: Chemistry and Biology, Journal of Applied Chemistry, 2013, 9, Article ID 621459, http://dx.doi.org/10.1155/2013/621459.
20. Gangwar P, Santhalingam K, Georgeb S, Chandranb M, Gururagavan M, Molecular Docking Studies of Novel 3-Substitued Phenyl-2-(furan-2-yl)-4H- Chromen-4-ones as Inhibitors of Interleukin-13 for Asthma, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(3), 2011, 1011-1014.

Source of Support: Nil, **Conflict of Interest:** None.