Research Article

Target related *in silico* analysis of Bergenin and tuberculosis management

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ABSTRACT

Introduction and Aim: Tuberculosis (TB) is a global health concern, claiming two million lives every year. Although an oldest known human infectious disease, researcher is falling short of giving out an effective and reliable vaccine or therapy. The current antimycobacterial drugs include Isoniazid, Ethambutol, Rifampicin and Pyrazinemamide available in market, but most of these are known to have certain adverse effects. Hence there is an increase in demand for natural products with anti-tuberculosis activity with no or limited side effects. Indian traditional systems of medicine have a plethora of promising plants for treatment of tuberculosis, of which *Bergenin* is the most well established and extensively used compound. The main aim of this research was to investigate the role of Bergenin as an anti-tuberculosis agent with the help of *in-silico* analysis and protein interaction studies.

Materials and Methods: In the present study 04 known 3-dimensional crystallized anti-tubercular drug target is considered and retrieved from PDB. Drug Isoniazid, Ethambutol, Rifampicin, Pyrazineamide and phytochemical Bergenin were retrieved, sketched and geometrically optimized. Molecular docking is carried to understand the binding mode and its core interactions. ADMET properties were calculated in assessment of the toxicity. Protein-protein interactions and enrichment analysis is carried out to understand the biological process involved with rpsA protein.

Results: In the present study other than Rifampicin, Bergenin reported with better binding energy and similar pharmacophoric interaction pattern as compared to all the 04 indigenous inhibitors. The PPI network and enrichment analysis predicts the plausible biological process involved with rpsA protein and can be further targeted in treatment of tuberculosis.

Conclusion: The results showed that Bergenin was better than and competent with the existing drugs and can be used as an anti-tuberculosis agent if studied *in-vitro* and *in-vivo* for its activity.

Keywords: Tuberculosis; Bergenin; *in silico*; proteomics

INTRODUCTION

Tuberculosis (TB) is a world-wide concern, costs multi-million lives each year (1). Being the most primitive known human infectious disease but, still lack of effective and decisive vaccine or therapy. Bacillus Calmette Guerin (BCG) developed in 1921 is the only known vaccine, but fails to protect against adult pulmonary TB (2).

Current line of therapy for TB, adopts DOTS (Directly Observed Treatment Short-course), which involves the use of multiple antibiotics and a lengthy regimen (3, 4). This may attract significant risk for the future generation to be drug resistant. It has been reported that, most of the countries are under threat by multiple drug-resistant (MDR) and extremely drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (M. tb) (3, 4). These antibiotics have potential to cause serious hepatic and immune problems and also tend to eliminate antigen-specific T cells (5, 6) which further may result in hyper susceptibility (6, 7). In view of this, there is continuous research is going on in search of an alternate, alternate equally or more potent drugs.

The current antimycobacterial drugs include isoniazid, ethambutol, rifampicin and Pyrazinemamide available in market, but most of these are known to have certain adverse effects. World Health Organization (WHO) and International Union against Tuberculosis and Lung Disease (IUATLD) recommend the replacement of single-drug preparations by fixed-dose combination

formulations (FDCs) as the primary treatment for tuberculosis (8). This recommendation can be justified with a simple approach of delivering the correct number of drugs at the correct dosage. This is done by combining all the drugs in a single tablet (8), this also has an advantage of altering number of tablets as per body weight of the patient. This ensures complete treatment without need for calculation of dose.

Rifampicin acts on RNA pol activity in the susceptible cells; it shows no interaction with mammalian enzymes (9) and at therapeutic levels, it has demonstrated bactericidal activity against both intracellular and extracellular *Mycobacterium tuberculosis* organisms (9). On other hand, isoniazid kills actively growing tubercle bacilli by inhibiting the biosynthesis of mycolic acids which are major components of the cell wall of *M. tuberculosis* (10,11). Ethambutol diffuses into actively growing *M. tuberculosis* such as tubercle bacilli and inhibits the synthesis of one or more metabolites, thus causing impairment of cell metabolism, arrest of multiplication, and cell death (12) . Whereas, the exact mechanism of action by which pyrazinamide inhibits the growth of *M. tuberculosis* organisms is not known. *In vitro* and *in vivo* studies have demonstrated that pyrazinamide is only active at a slightly acidic pH (pH 5.5; 13-15).

Hence there is an increase in demand for natural products with anti-tuberculosis activity with no or limited side effects. Indian traditional systems of medicine have a plethora of promising plants for treatment of tuberculosis, of which *Bergenin* is most well established and extensively used. Not much information is available on the mechanism of action of the drug Bergenin in treatment of tuberculosis. Currently most of the traditional drugs were investigated and studied using modern scientific methods including computational modeling approaches. As a result traditional medicines/ ethnomedicines are widely used all over the world and being reevaluated using extensive research on the base material from the plants and on their therapeutic principles in order to determine their actual efficacies and any limitations on the safe use of these drugs and the results of such research are expected to widen the scope of their future use (16).

MATERIALS AND METHODS

Protein preparation

The three-dimensional crystalline structures of 04 targeted proteins were retrieved from the Protein Data Bank (http://www.rcsb.org/). The retrieved protein PDB IDs 1W6F (17), 3PTY (18), 5KOX (19) and 4NNI (20) were in complex with indigenous inhibitors such as – Isoniazid, Ethambutol, Rifampicin and Pyrazineamide. The coordinates of the structures were complexed with water molecules and other atoms which are responsible for increased resolution and therefore the water molecules and hetatoms were removed using discovery studios and saved in .pdb format.

Ligand and energy minimization

The 2D and 3D chemical structures of ligand (Isoniazid, Ethambutol, Rifampicin and Pyrazineamide) and Bergenin available on PubChem database were retrieved (21). All the smile notation formats obtained from PubChem were converted into the .mol file using Chem Sketch (22). Ligand was energy minimized using universal force filed in Argus lab and save in .pdb format for further process (23). Further the ligand is added with gastegier charges and made flexible using Auto dock tool and saved in pdbqt format.

Binding pocket analysis

Binding pocket/cavity plays a key role in the binding interaction between target receptor and ligand of any drug design process. The binding pocket/ cavity in all the 04 target proteins were considered as default with respect to their complexed inhibitors, and the Pharmacophoric selection is carried out using Auto Dock tool (24) and the cavity is consists of all the listed pharmacophores from the crystallized data. Finally, the protein with the selected cavity size and dimension is saved in pdbqt format. In 3PTY.pdb, the active site pharmacophore considered was ASN740, ASP1051, ASP1052, and ARG1055.

Ligand-protein docking

The docking is carried out using Autodock Vina tool (25), 10 conformation of ligands for each complex are generated and the complex with the lowest energy is considered.

Conformation and validation of docking process is the most important point in the *in-silico* docking studies. In this case here we have validated the docking process by interacting the indigenous inhibitors of the target protein and comparing the pharmacological interaction with the crystallized data represented on the pdb website with respect to least energy complex data generated. Later we have used the same cavity and docked the Bergenin and listed the pharmacological interaction for the least energy generated complex.

ADMET

Here we calculated ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) properties for all the four selected molecules using Swiss ADME (26) and Admet SAR (27), online servers to predict the significant pharmaceutical and toxicological properties.

Physiochemical properties such as molecular formula, molecular weight, Number of heavy atoms, Number of aromatic heavy atoms, number of

rotatable bonds, number of hydrogen bond donor and acceptor, Total polar surface area, Lipophilicity values, water solubility, Pharmacokinetics parameter and Lipinski violations were calculated from SwissADME server Table 1.

Caco-2 cell permeability, brain/blood barrier, human intestinal absorption, carcinogens and acute oral toxicity were calculated using AdmetSAR server Table 2.

Protein-protein interaction network prediction for rpsA from H37RV

Initially we performed a protein-protein interaction network prediction for rpsA protein of *Mycobacterium tuberculosis* strain H37Rv using String database (28), the active interaction sources were considered as follows: text-mining, experiments, databases, Co-expression, Neighborhood, Gene-fusion and Co-occurrence. A highest confidence score is kept as a cut-off score with 0.900. Simultaneously string also performs enrichment analysis for biological process, molecular function and cellular components; here we considered only biological process and molecular function.

RESULTS

Numerous ADMET properties were calculated for all the selected 05 molecules, except rifampicin all are soluble and safely passed the Lipinski violations Table 1. Whereas all have exhibited an acute oral toxicity, Table 2.

As the docking simulations were carried out among the active site of 04 target protein pdb id: 1W6F, 3PTY, 5KOX and 4NNI with their indigenous inhibitor Isoniazid, Ethambutol, Rifampycin, Pyrazinemanide and compared against Bergenin using Autodock vina tool. The docking score and pharmacological interactions were listed in table 3. The docking score of Bergenin scored from -4.8 Kj/mol with pdb-id 4NNI (Ribosomal protein S1), -5.7 KJ/mol with 3PTY.pdb C-terminal extracellular domain of *Mycobacterium tuberculosis*, -5.8 with 1W6F.pdb, Arylamine N-acetyltransferase from *Mycobacterium smegmatis* and -7.8 with 5KOX rifampicin monooxygenase (Figure 1 and 2), Table 3.

Table 1. Physicochemical property of drug compounds and Bergenin

Properties	Molecule				
	Isoniazid	**Ethambutol**	**Rifampicin**	**Pyrazineamide**	**Bergenin**
Formula	$C_6H_7N_3O$	$C_{10}H_{24}N_2O_2$	$C_{43}H_{58}N_4O_{12}$	$C_5H_5N_3O$	$C_{14}H_{16}O_9$
MW	137.14	204.31	822.94	123.11	328.27
#Heavy atoms	10	14	59	9	23
#Aromatic heavy atoms	6	0	6	6	6
#Rotatable bonds	2	9	5	1	2
#H-bond acceptors	3	4	14	3	9
#H-bond donors	2	4	6	1	5
MR	35.13	58.11	230.18	30.13	72.8
TPSA	68.01	64.52	216.66	68.87	145.91
Consensus Log P	-0.35	0.6	2.23	-0.37	-0.72
Ali Class	Very soluble	Very soluble	Poorly soluble	Very soluble	Very soluble
Silicos-IT LogSw	-1.64	-2.14	-4.59	-1.2	0.09
Silicos-IT class	Soluble	Soluble	Moderately soluble	Soluble	Soluble
log Kp (cm/s)	-7.63	-7.6	-8.1	-7.48	-8.99
Lipinski #violations	0	0	3	0	0
Bioavailability Score	0.55	0.55	0.17	0.55	0.55

Table 2. Toxicity prediction using ADMET SAR

Molecule		Isoniazid	Ethambutol	Rifampicin	Pyrazineamide	Bergenin
Blood-Brain Barrier	Result	+	-	-	+	-
	Probability	0.9895	0.7803	0.9803	0.9745	0.8869
Human Intestinal Absorption	Result	+	+	+	+	+
	Probability	0.9892	0.9157	0.7721	0.9813	0.5078
Caco-2 Permeability	Result	+	+	-	+	-
	Probability	0.6959	0.5000	0.8957	0.7222	0.8679
Carcinogenicity (Three-class)	Result	warning	NR	NR	NR	NR
	Probability	0.4786	0.5818	0.5419	0.7191	0.6881
Acute oral toxicity	Result	III	III	III	III	III
	Probability	0.8032	0.8256	0.6698	0.8145	0.7058
Rat Toxicity LD50 mol/kg	Result	2.0713	2.2797	2.7018	1.8145	2.1775

Table 3. Molecular docking output

Receptor Pdb-id	Drug / Ligand	Hydrogen bonds	Pi-interactions	Docking energy
1W6F Arylamine N-acetyltransferase from Mycobacterium smegmatis	Isoniazid	His 110, Gly 129	Cys 70, Val 95, Phe 130, Phe 204	-5.3
	Bergenin	Phe 38, Thr 109	Val 95, His 203	-5.8
3PTY C-terminal extracellular domain of Mycobacterium tuberculosis EmbC	Ethambutol	Asn 740, Asp 1051, Arg 1055	-----	-3.5
	Bergenin	Asn 740, Arg 1055	Leu 1049, Asp 1051	-5.7
5KOX rifampicin monooxygenase	Rifampicin	Arg 43, Gly 285, FAD 501	His 46, Phe 69, Val 71, Val 93, Leu 176, Arg 201, Ile 215, Phe 256, Pro 283, FAD 501	-13.9
	Bergenin	Arg 196, FAD 501	Pro 283	-7.8
4NNI the ribosomal protein S1 of Mycobacterium tuberculosis	Pyrazineamide	Arg 357	Phe 307	-2.7
	Bergenin	His 322, Arg 357	Phe 307	-4.8

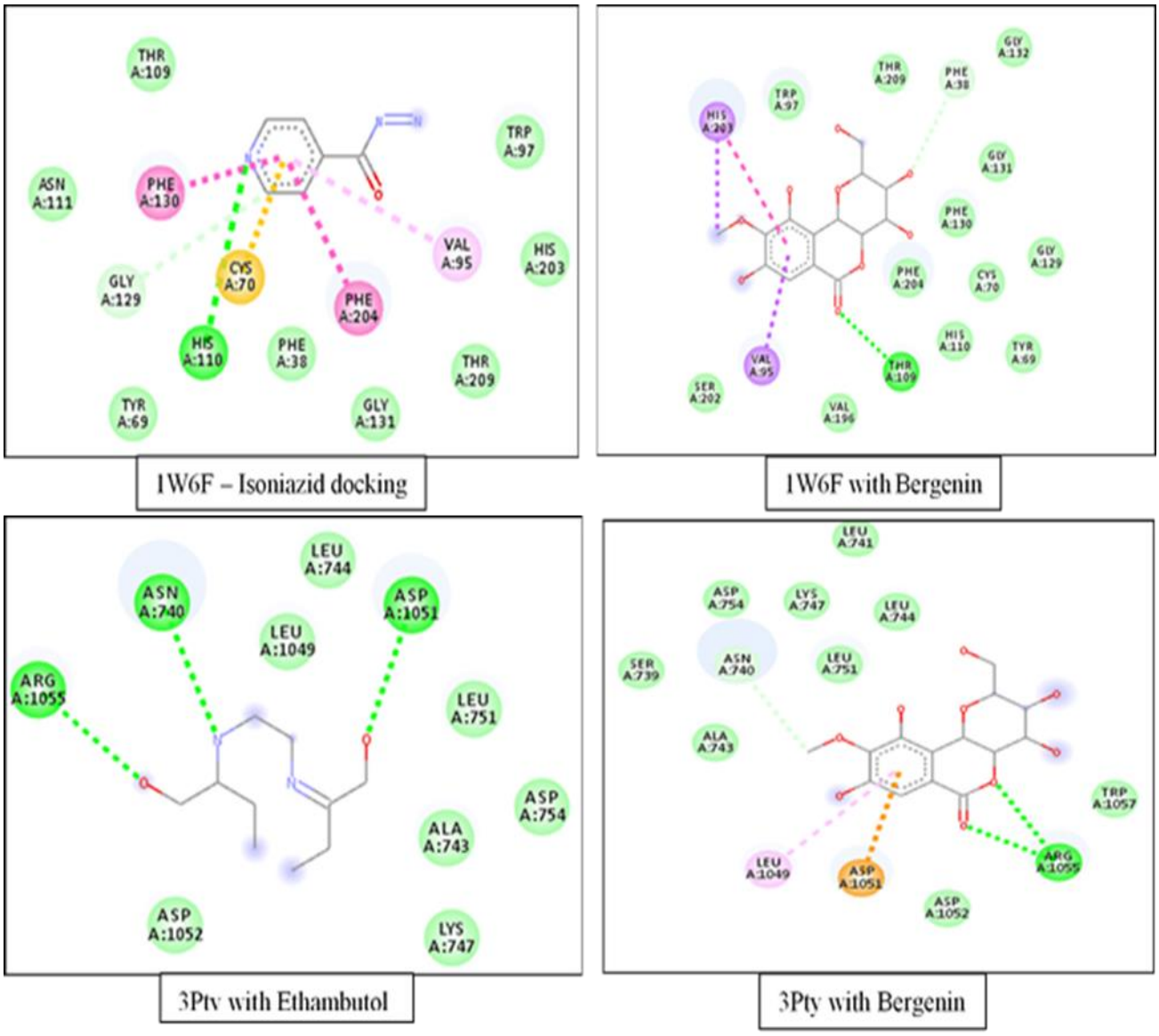

Fig. 1: 2D interactions of the ligand with the protein

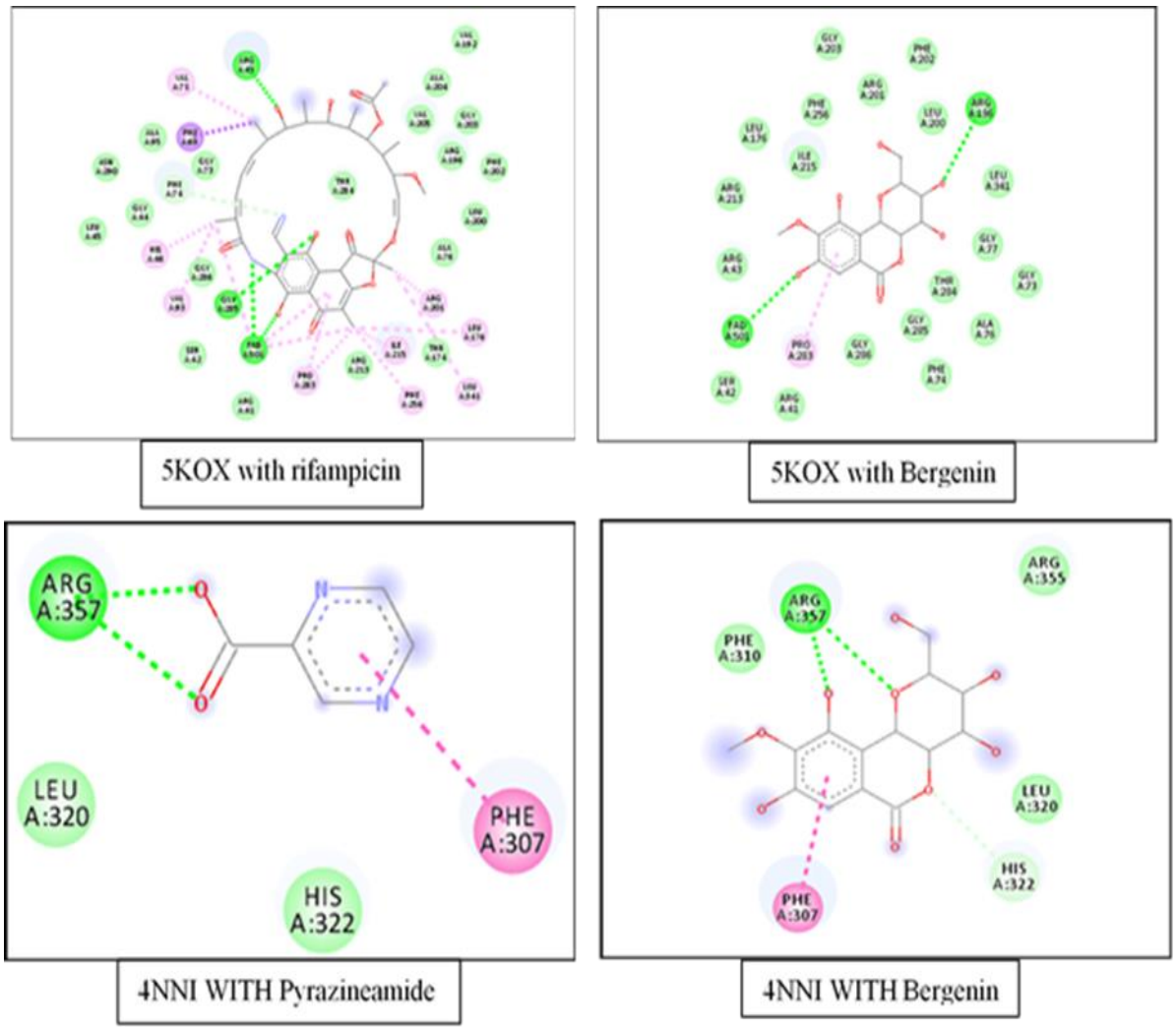

Fig. 2: 2D interactions of the ligand with the protein

Protein – protein interaction network analysis

The PPIN for rpsA depicted a strong interaction with 10 ribosomal protein, rplA, rplJ, rplU, rplW, rpmI, rpsB, rpsD, rpsG, rpsJ and rpsL within organism: Mycobacterium tuberculosis H37Rv (Figure 3).

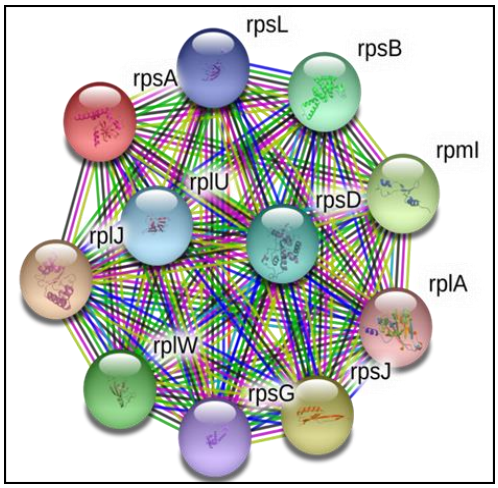

Fig. 3: Protein-protein interaction network of rpsA protein

Enrichment analysis

PPI network predicts the plausible biological process involved with rpsA protein in growth and other interactive proteins are involved in translation, peptide metabolic process, growth, ribosome biogenesis, ribosomal large subunit biogenesis, ribosome assembly and regulation of translation (Table 4).

The enriched molecular function describes the rpsA plausible role in RNA binding and other list of

proteins involved in structural constituent of ribosome, RNA binding, rRNA binding and tRNA binding (Table 5). In cellular component analysis rpsA was found to be placed in ribosome, cytosol, cell wall and plasma membrane. The other interactive proteins were involved in ribosome, ribosomal subunit, cytosolic ribosome, small ribosomal subunit, cytosol, cytosolic large ribosomal subunit, cell wall and plasma membrane (Table 6).

Table 4: rpsA protein enrichment analysis for biological process

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (labels)
GO:0006412	Translation	10	96	1.01E-13	rplA,rplJ,rplU,rplW,rpmI,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0006518	peptide metabolic process	10	107	1.18E-13	rplA,rplJ,rplU,rplW,rpmI,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0040007	Growth	9	541	3.14E-06	rplJ,rplU,rplW,rpsA,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0042254	ribosome biogenesis	4	33	8.25E-06	rplA,rplJ,rplW,rpsG
GO:0042273	ribosomal large subunit biogenesis	2	6	0.00062	rplA,rplW
GO:0042255	ribosome assembly	2	9	0.0011	rplW,rpsG
GO:0006417	regulation of translation	2	15	0.0023	rplA,rpsD

Table 5: rpsA protein enrichment analysis for molecular function

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (labels)
GO:0003735	structural constituent of ribosome	10	56	7.31E-17	rplA,rplJ,rplU,rplW,rpmI,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0003723	RNA binding	9	100	9.60E-13	rplA,rplJ,rplU,rplW,rpsA,rpsD,rpsG,rpsJ,rpsL
GO:0019843	rRNA binding	7	40	1.45E-11	rplA,rplJ,rplU,rplW,rpsD,rpsG,rpsL
GO:0000049	tRNA binding	3	19	2.82E-05	rplA,rpsG,rpsL

Table 6: rpsA protein enrichment analysis for cellular component

#term ID	term description	observed gene count	Back ground gene count	false discovery rate	matching proteins in your network (labels)
GO:0005840	Ribosome	11	61	7.38E-19	rplA,rplJ,rplU,rplW,rpmI,rpsA,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0044391	ribosomal subunit	9	49	2.95E-15	rplA,rplJ,rplW,rpmI,rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0022626	cytosolic ribosome	6	42	2.11E-09	rplA,rplJ,rplW,rpmI,rpsB,rpsG
GO:0015935	small ribosomal subunit	5	22	8.14E-09	rpsB,rpsD,rpsG,rpsJ,rpsL
GO:0005829	cytosol	9	416	1.04E-07	rplA,rplJ,rplW,rpmI,rpsA,rpsB,rpsD,rpsG,rpsJ
GO:0022625	cytosolic large ribosomal subunit	4	26	1.16E-06	rplA,rplJ,rplW,rpmI
GO:0005618	cell wall	7	529	0.00017	rplA,rplJ,rpsA,rpsB,rpsD,rpsG,rpsJ
GO:0005886	plasma membrane	9	1091	0.00029	rplA,rplJ,rplU,rplW,rpsA,rpsB,rpsD,rpsG,rpsJ
GO:0022627	cytosolic small ribosomal subunit	2	15	0.0009	rpsB,rpsG

DISCUSSION

Except for rifampicin which showed better binding affinity than bergenin, all the other molecules had lower binding affinity indicating a better interaction of bergenin with the *Mycobacteruim* proteins as against existing TB drugs (figure 1 and 2). Phytochemical extracts from *Costus speciosus*, *Cymbopogon citratus*, and *Tabernaemontana coronaria* exhibited the highest inhibitory activity against *M. tuberculosis* H37Rv (29). Phytochemicals including methyl chavicol and caryophyllene were reported as antimycobacterial compounds (30). Kumar et al, has suggested that bergenin is a potent immunomodulatory agent that could be further explored as a potential adjunct to TB therapy (31). All the associated protein from PPIN based analysis including rpsA reported in Ribosome pathway KEGG-id: mtu03010. The PPIN and enrichment analysis reveals the all the possible role and activeness of rpsA protein in the above stated biological process, molecular function and cellular component. Hence here we conclude with our study that in the process of inhibition of rpsA protein might affect either or all the above stated process and function.

CONCLUSION

Bergenin shows promising binding results as compared to the existing drugs for tuberculosis. These results can be further validated by conducting wet lab analysis to develop a better lead. An herbal drug may have less adverse or toxic effects. The protein network analysis shows the interaction of different proteins along with the central role of rspA protein in the molecular functioning of tuberculosis. These pathways can be further targeted to control tuberculosis treatment.

Definitively bergenin structure would act as a lead scaffold for developing lead analogs to optimize the antitubercular activity furthermore based on the protein network analysis and topology various receptors can be considered for analyzing as well as optimizing berginin derivatives antitubercular activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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