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Research Article

QUERCETIN, BERGAPTEN AND BARBERINEB AS ANALOGUES OF RIFAMPICIN AND ISONIAZID SCREENED IN-SILICO FROM HERBAL PLANTS OF UTTARAKHAND FOR THE TREATMENT OF TUBERCULOSIS (TB)

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ABSTRACT: *Mycobacterium tuberculosis* (MBT) has become resistant to most of the drugs that were being used for the treatment of tuberculosis (TB). With this increase in the resistance of MBT towards almost all drugs, the need for new drugs that can inhibit the activity of MBT and treat millions of patients worldwide is one of the major reasons of concern. *In-silico* analysis of phytochemicals has provided the potential to screen numerous phytochemicals. By employing a Molecular docking software iGEMDOCK, 3 possible analogues of Rifampicin and Isoniazid which are known inhibitors of MBT were screened based on the comparison of their binding energies to the known inhibitors. Isoniazid inhibits Enoyl-acyl carrier protein (InhA), which is responsible for the synthesis of type II fatty acid and Rifampicin inhibits the protein mycobacterium dependent RNA-polymerase required for RNA synthesis. Quercetin, Bergapten and Barberineb are the chemical compounds which can be the best possible analogues of Rifampicin and Isoniazid as they bind to their target proteins with the same binding energy as the two known inhibitors.

Keywords: *Mycobacterium tuberculosis* (MBT), iGEMDOCK, Rifampicin, Isoniazid, Quercetin, Bergapten and Barberineb.

Introduction

Tuberculosis (TB) is the most prevalent air-borne disease, infecting millions of people worldwide each year. It has been estimated that each year 10 million people are diagnosed with TB and 2 million patients die from the disease every single year (Glickman *et al*., 2006). Various drugs had been developed for the cure of TB such as Rifampicin (R) and Isoniazid (I), and they are also known as the first-line drugs for anti-TB therapy (Orienstein *et al*., 2009). The resistance of *Mycobacterium tuberculosis* (MTB) to R, I and other drugs is referred to as Multi-Drug Resistant Tuberculosis (MDR-TB) and this further exaggerated the problem as most of the drugs were ineffective (Johnston *et al*., 2009). After MDR-TB, various other drugs such as fluoroquinolone, kanamycin, amikacin and capreomycin had been developed to treat MDR-TB but to everyone's dismay, it too went in vain and led to the development of new infectious strains called Extensively-Drug resistant TB (XDR-TB) (Ahmad *et al.*, 2013). The ineffectiveness of drugs to MTB had had a very bad impact on the lives of various individuals with no option than to surrender to the disease (Van *et al*., 2013).

The largest number of MDR-TB cases in the World has been reported in India (WHO REPORT. 2012). Rifampicin and Isoniazid were the first line drugs used for TB therapy and it had proved very beneficiary until the development of MDR and XDR-TB strains (Dye *et al*., 2006, Gandhi *et al*., 2006 and Zignol *et al*., 2006). Due to the development of mutant strains, different new drugs are been manufactured which hold promises to treat patients with TB with least side-effects and longer period of susceptibility towards the bacteria. Nowadays, researchers have developed a keen interest in discovering novel compounds from natural compounds which would be cost effective in developing as well as efficient in inhibiting its target enzyme. Some drugs currently in its trial phases are Moxifloxacin and gatifloxacin (Phase III of its clinical trials), PA-824 and TMC207 (Phase II) and SQ109, AZD5847 and linezolid (Phase I) (Matsumoto *et al*., 2006).

In this study, our focus was on the screening of herbal compounds of Uttarakhand with the potential of treating TB by acting as analogues of the first line drugs Rifampicin and Isoniazid. The screening of herbal compounds from different plant species was done with a Bioinfomatic approach. 112 species of plants from different regions of Uttarakhand were screened to identify phytochemicals showing inhibitory characteristics towards MBT. This screening was done using software iGEMDOCK (Generic Evolutionary Method for Molecular docking), a Molecular Docking software (http://gemdock.life.nctu.edu.tw/dock/igemdock.php). iGEMDOCK generates protein-compound interaction profiles of Van Der Waal's (V), Hydrogen-Bonding (H) and Electrostatic (E) interactions. This molecular docking method screens molecules on the basis of their binding energies and ligand binding efficiency and thus predicts the role of the compound as an inhibitor of our desired protein (Kumar *et al*, 2014). Based on these principles and the

structure of compounds, iGEMDOCK analyzes the interactions between the proteins and its most favorable ligands by calculating the binding energy or fitness energy with which each ligand binds to its respective protein's active site (Kai-Cheng *et al*., 2011). This approach screens a large number of phytochemicals by analyzing the molecular interactions between the desired compounds. iGEMDOCK sequentially reads the target coordinates of the protein and ligand molecule atoms and analyzes its molecular interactions by executing flexible docking interactions (Arun *et al*., 2012). Therefore, iGEMDOCK provides an efficient and time saving approach towards the screening of herbal components for its modulatory effects on different proteins of interest.

Materials and Methods

This study was carried out to screen herbal compounds as analogues of Rifampicin and Isoniazid. The screening process was done using a molecular docking software iGEMDOCK. iGEMDOCK calculates the binding energies (B) of the ligand to its protein molecules by analyzing the electrostatic interaction(E), Van der Waals force(V) and hydrogen bonding(H). The binding energy or fitness energy determines how accurately or the strength with which a ligand binds to the receptor on the surface of its target protein molecule (Jing-Moon *et al*., 2004). By understanding these interactions, it was possible to identify our compound of interest.

iGemdock requires the PDB (Protein Data Bank) structural format of the target protein (Binding site) molecule and the MOL2 structural format of the herbal compound (ligand).

Preparing Binding Site

The PDB (Protein Data Bank) structural formats of the target proteins were obtained from RSCB protein data bank. The PDB I.D of Enoyl-acyl carrier protein (InhA) to which Isoniazid (known inhibitor) binds and inhibits it by inhibiting fatty acid synthesis of MTB and Mycobacterium Dependent RNA Polymerase to which Rifampicin binds, inhibits MBT by inhibiting its RNA synthesis (Nanashima *et al*., 2012), were generated from RSCB PDB and are given below: A snapshot of the RSCB webpage has been given in fig 1(A).

- Mycobacterium dependent RNA Polymerase: PDB I.D = **4KBJ**
- Enoyl-Acyl Carrier Protein (InhA): PBD I.D = **2H71**

Preparing Ligand

The MOL2 structural formats of all the 30 herbal components were generated from the database ZINC AC. The MOL2 formats of Rifampicin and Isoniazid, known inhibitors of MBT were also generated and their binding energies were used as controls. The Mol2 I.D of the ligand compounds are given below: A snapshot of ZINC AC webpage has been given in fig 1(B).

1. Myrecitin (ZINC I.D = 14436449)
2. Kaempherol (ZINC I.D = 3869765)
3. Quercetin (ZINC I.D = 3869685)
4. Betulinic (ZINC I.D = 35494088)
5. Anthocyanin (ZINC I.D = 1670024)
6. Delphinidin (ZINC I.D = 38601496)
7. Malvidin (ZINC I.D = 15657701)
8. Petunidin (ZINC I.D = 3954302)

9. Ellagic (ZINC I.D = 3872446)
10. Gallic Acid (ZINC I.D = 1504)
11. Barberineb (ZINC I.D = 3779067)
12. Oxyberberine (ZINC I.D = 1604019)
13. Berbamine (ZINC I.D = 30726840)
14. Palmatine (ZINC I.D = 608233)
15. Sitosterol (ZINC I.D = 4095717)
16. Betulin (ZINC I.D = 3978650)
17. Lupenone (ZINC I.D = 79669733)
18. Skimmianine (ZINC I.D = 35525)
19. Lupeol (ZINC I.D = 4081455)
20. Citral (ZINC I.D = 1529208)
21. Marmin (ZINC I.D = 14587259)
22. Eugenol (ZINC I.D = 1411)
23. Bergapten (ZINC I.D = 57731)
24. Bergaptol (ZINC I.D = 5842977)
25. Lansterol (ZINC I.D = 3870053)
26. Oleic Acid (ZINC I.D = 6845860)
27. Cedrol (ZINC I.D = 3978625)
28. Phytosterol (ZINC I.D = 6393492)
29. Squalene (ZINC I.D = 6845904)
30. Luteolin (ZINC I.D = 18185774).
31. Isoniazid (ZINC I.D = 54853, control 1)
32. Rifampicin (ZINC I.D = 13292461, control 2).

Docking module

Now, with the structural PDB and Mol2 formats of each compound generated, the molecular docking of the proteins and its suitable ligands was carried out using iGEMDOCK. First, we analyzed and calculated the molecular interactions of the known proteins of MBT with both the control compounds. Then, each of the 30 experimental herbal compounds was docked with 4KBJ and 2H71 respectively. The molecular docking using iGEMDOCK generated the binding energies of all the 30 herbal compounds. This binding energy describes how fit a ligand binds to the receptor of its target protein and an image showing this interaction is also generated. The generated image displays the conformation of the bound ligand to its protein. The images have been shown in fig 2.

Results and Discussions

Out of 30 herbal compounds which were tested against 4KBJ and 2H71, 57731(Bergapten74.91) and 3779067 (Barberineb74.83) were screened as the best possible analogues of Isoniazid and 3869685(Quercetin68.41) was screened as the best possible analogue of Rifampicin. Bergapten is a compound isolated from *Santalum album* (Chandan), Barberineb from *Berberis aristata* (Kirmod) and Quercetin from *Syzygium cumini* (Jamun). Quercetin has also been reported earlier in the treatment of TB (Yoshino *et al*., 1958 and Lakovlev *et al*, 1986). Rifampicin binds to Mycobacterium dependent RNA polymerase with a binding energy of -68.6 and Isoniazid binds to InhA with a binding energy of -74.49. Therefore, Quercetin is the best possible analogue of Rifampicin as it binds to Mycobacterium dependent RNA polymerase with a binding energy of -68.41. Bergapten and Barberineb bind to InhA with binding energies of -74.91 and -74.83 respectively and are therefore the best analogues of Isoniazid, which has a binding energy of -74.49. The binding energies of the phytochemicals have been listed in Tables 1 and 2.

Conclusion

The resistance of MBT to so many drugs and its drastic rise in infection over

the past two decades has built a concern as to which chemical compound is capable of inhibiting MBT. Manual screening is an efficient but tedious and time consuming process; therefore *in-silico* approach has been used to screen large number of compounds in much less time. iGEMDOCK is an efficient molecular docking software and with its use, 30 herbal compounds isolated from different species of plants grown in Uttarakhand were screened for their potential to inhibit MBT and out of these 30 compounds, 3 chemical compounds namely Quercetin, Bergapten and Barberineb were selected based on their molecular docking interactions to be the best possible analogues of Rifampicin and Isoniazid, known inhibitors of MBT. The argument that MBT is already resistant to Rifampicin and Isoniazid and so it will also be resistant to shortlisted components has been discussed. In this regard the argument that the mechanism of action of these compounds on target might be at different target site or altogether at a different target has been made. These phytochemicals hold the potential to inhibit MBT by inhibiting its fatty acid and RNA synthesis. More research on these phytochemicals is being carried out in the wet lab.

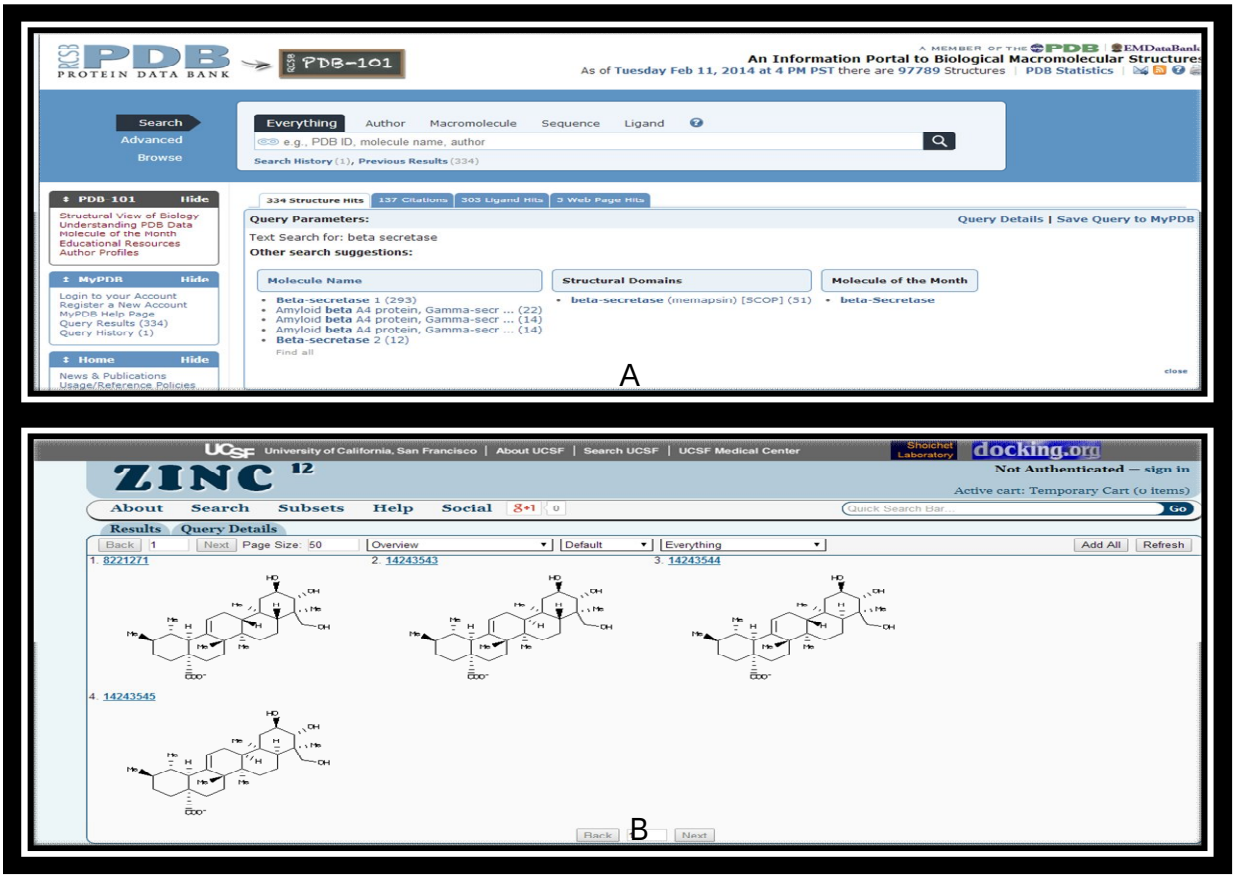

Fig. 1. Snapshot of the web pages of A) RSCB PDB and B) ZINC AC.

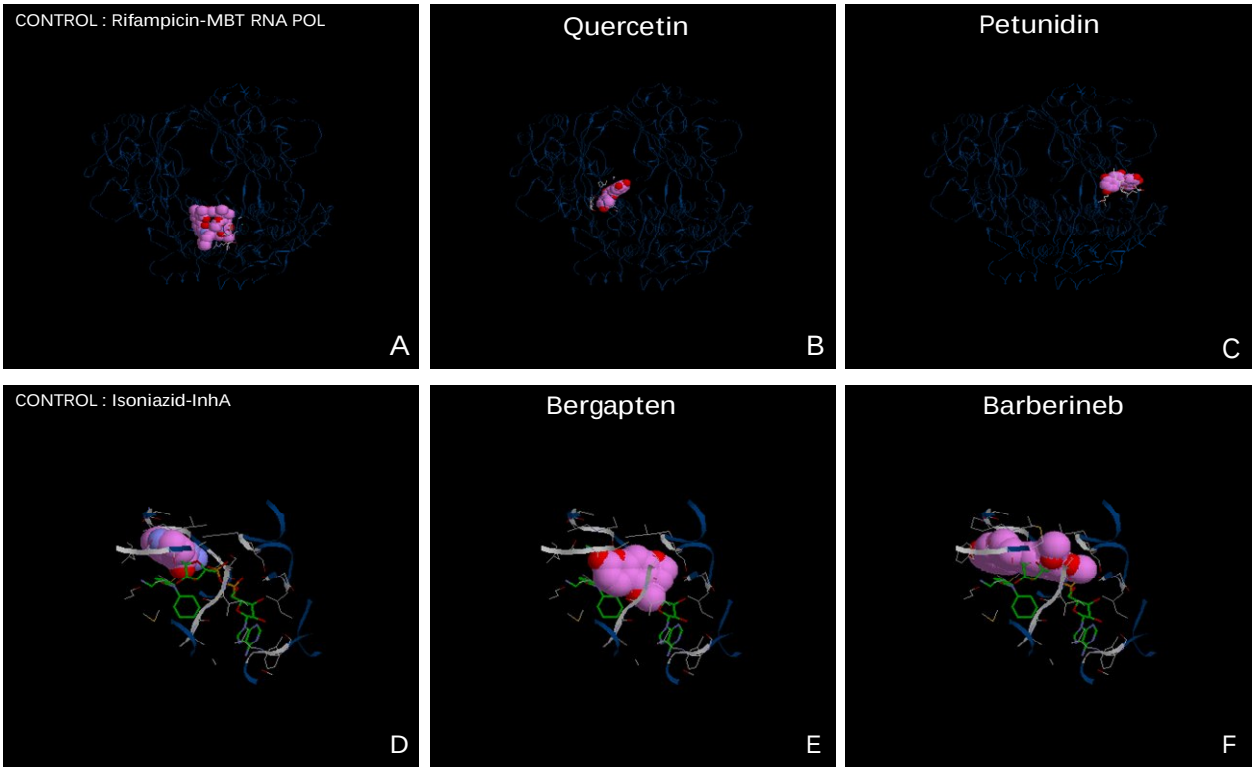

Fig. 2. Docking results of A) Rifampicin-MBT RNA POL, B) Quercetin-MBT RNA POL, C) Petunidin-MBT RNA POL, D) Isoniazid-InhA, E) Bergapten-InhA and F) Barberineb-InhA. The red and pink coloured molecular structures represents the bound phytochemical on the blue coloured ribbon structure representing the proteins MBT RNA POL (A, B&C) and InhA (D, E&F).

Table 1: Binding energies of 30 docked phytochemicals with InhA.

S.NO.	PLANT COMPOUND/ DRUG	BINDING ENERGY	ELECTOSTATIC FORCE	VAN DER WAALS FORCE	HYDROGEN BONDING
CONTROL 1	**ISONIAZID**	**-74.49**	**0**	**-45.5**	**-29**
1	Myrecitin	-83.33	0	-54.53	-31.81
2	Kaempherol	-95.86	0	-74.03	-21.84
3	Quercetin	-80.71	0	-67.89	-12.82
4	Betulinic	-62.05	0	-50.44	-11.61
5	Anthocyanin	-71.3	0	-71.3	0
6	Delphinidin	-79.18	0	-66.49	-12.69
7	Malvidin	-87.85	0	-79.16	-8.69
8	Petunidin	-91.66	0	-64.42	-27.24
9	Ellagic	-102.83	0	-86.61	-16.22
10	Gallic Acid	-70.98	0	-56.37	-14.61
11	**Barberineb**	**-74.83**	**0**	**-63.78**	**-11.06**
12	Oxyberberine	-81.26	0	-74.24	-7.02
13	Berbamine	-53.18	0	-44.95	-8.23
14	Palmatine	-80.52	0	-78.02	-2.5
15	Sitosterol	-71.76	0	-67.01	-4.75
16	Betulin	-59.95	0	-55.2	-4.74
17	Lupenone	-60.21	0	-60.21	0
18	Skimmianine	-80.84	0	-72.8	-8.04
19	Lupeol	-63.14	0	-63.14	0
20	Citral	-52.7	0	-46.71	-5.99
21	Marmin	-78.57	0	-62.68	-15.88
22	Eugenol	-61.98	0	-55.30	-6.68
23	**Bergapten**	**-74.91**	**0**	**-62.96**	**-11.94**
24	Bergaptol	-76.1	0	-59.44	-16.66
25	Lansterol	-60.27	0	-60.27	0
26	Oleic Acid	-60.83	0	-60.55	-0.19
27	Cedrol	-57.67	0	-50.69	-6.98
28	Phytosterol	-55.88	0	-47.57	-8.31
29	Squalene	-62.87	0	-62.87	0
30	Luteolin	-86.54	0	67.2	-19.34

TABLE 2: Binding energies of 30 docked phytochemicals with MBT RNA polymerase.

S.NO.	PLANT COMPOUND /DRUG	BINDING ENERGY	ELECTOSTATIC FORCE	VAN DER WAALS FORCE	HYDROGEN BONDING
CONTROL 2	**RIFAMPICIN**	**-68.6**	**0**	**-61.1**	**-7.5**
1	Myrecitin	-76.95	0	-52.45	-24.5
2	Kaempherol	-73.57	0	-55.57	-18
3	**Quercetin**	**-68.41**	**0**	**-45.01**	**-23.4**
4	Betulinic	-61.35	0	-58.46	-1.3
5	Anthocyanin	-57.12	0	-57.12	0
6	Delphinidin	-95.89	0	-65.95	-29.94
7	Malvidin	-71.25	0	-57.59	-13.66
8	Petunidin	-67.42	0	-57.49	-9.93
9	Ellagic	-70.41	0	-56.06	-14.34
10	Gallic Acid	-62.02	0	-47.73	-13.15
11	Barberineb	-58.78	0	-55.28	-3.5
12	Oxyberberine	-78.13	0	-74.63	-3.5
13	Berbamine	-54.8	0	-54.24	-0.56
14	Palmatine	-59.85	0	-59.85	0
15	Sitosterol	-61.28	0	-60.61	-0.67
16	Betulin	-60.48	0	-50.74	-9.74
17	Lupenone	-62.87	0	-62.87	0
18	Skimmianine	-78.88	0	-64.79	-14.09
19	Lupeol	-55.66	0	-55.02	-0.64
20	Citral	-38.3	0	-35.5	-2.5
21	Marmin	-73.63	0	-64.51	-9.12
22	Eugenol	-50.08	0	-43.58	-6.5
23	Bergapten	-58.23	0	-48.32	-9.91
24	Bergaptol	-62.04	0	-46.1	-15.94
25	Lansterol	-65.65	0	-62.36	-3.3
26	Oleic Acid	-51.26	-1.07	-50.19	0
27	Cedrol	-52.9	0	-48.86	-4.04
28	Phytosterol	-72.98	0	-72.98	0
29	Squalene	-64.5	0	-64.5	0
30	Luteolin	-72.12	0	-53.01	-19.11

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