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IN SILICO HERBAL BIOPROSPECTION TARGETING MULTI- DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

The aim of the present study is to select promising plants against multi-drug resistant *Mycobacterium tuberculosis,* by using a bioprospection model and further evaluation of the selection of herbals against the pathogen on the basis of molecular docking results. Statistical based approach was used that rule on matrices based aspect operating on numerical linear algebra. Percentage relevance, Weightage score, Coefficient of association based Binary score estimation, Fuzzy score calculation and optimization of the final results were used to select promising plants against the pathogen. By using Hex 6.12 software molecular docking was performed by targeting different physiological elements (Acyl-Co-A, Salicylate synthase, Ag-85, RNA binding protein and Transcription regulator) with major phytoconstituents of the selected plants. Six plants (*Vetiveria zizanoides; Solanum panduriforme; Piper nigrum; Zingiber officinale; Foeniculum vulgare;* and *Gardenia jasminoides)* were selected for their potent activity against *Mycobacterium tuberculosis.* Chemiinformatics study using molecular docking reveals that among all phytoconstituents 6-gingerol and paradol from *Zingiber officinale* have been found to be most potent against each target. The study presents a more promising approach of herbal selection against *Mycobacterium.tuberculosis*. The present study showed that the model provides a rationale based selection of plants against such pathogen. Further this study will curtail cost of herbal drug development as only promising plants will be taken up for further studies.

Keywords: *Mycobacterium tuberculosis,* Bioprospection, Molecular Docking.

INTRODUCTION

Tuberculosis (TB), a latent infection affecting nearly one third of world's population has already been aggravated with the emergence of its multi-drug resistant strains like Multi-drug Resistant *Mycobacterium tuberculosis* (MDR-*Mtb*) showing resistance against Isoniazid and Rifampicin.[1- 3] Prevalence of MDR-*Mtb* and issues with currently available drugs and drug resistance, etc., necessitates investigation of complementary and alternative therapies. Desirable therapeutic regimen should aim at lessening the duration of treatment, reducing side-effects and dosing schedule. Such emergence of resistance is linked to unhygienic conditions and improper use of antibiotics. [13] As per World Health Organization (WHO) Global Tuberculosis Report (2014) the average mortality rate attributed to drug resistant strain accounts for around 2.1 lakhs deaths as compared to 15 lakhs deaths in non-resistant counterpart. [4] Moreover, 3.7% of new TB patients globally suffer from such

multi-drug resistant strains. [2, 3, 5, 6] presently used countermeasures include combination of high doses of first-line and second-line antitubercular drugs. The associated side effects with such regimen like ototoxicity, hepatotoxicity, nephrotoxicity, hypersensitivity, agranulocytosis, etc., upon their long term usage limit their ultimate utility. [2, 7- 12] A study of the probable biochemical mechanism involved in imparting pathogenecity and drug resistance to *Mtb* is depicted in fig. 1.

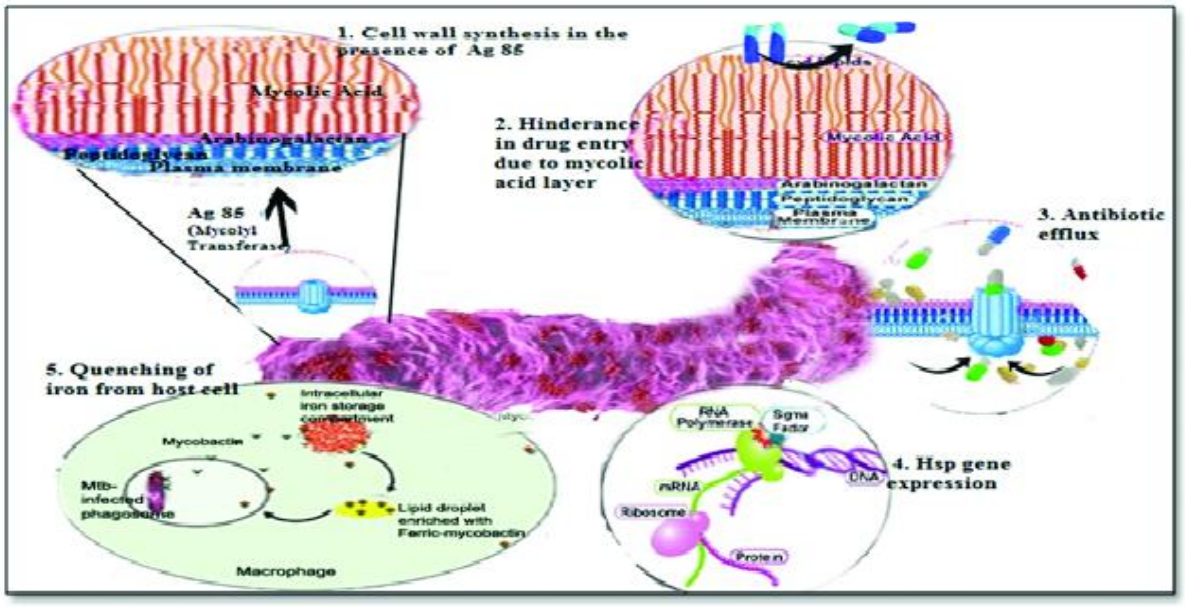

Figure 1: Mechanism of pathogenesis and resistance against antibiotics (1) Cell wall synthesis by addition of Mycolic acid carried out by Antigen 85; (2) Antibiotics do not get enter because of Mycolic acid in the cell wall; (3) Antibiotics pumped out by efflux pump in the cell wall; (4) Expression of Heat shock proteins under stressful physiological environment initiated in the presence of sigma factor; (5) *Mtb* inside phagosome of host cell quench iron in order to sustain their own life.

There is no clinically proven alternative herbal therapy available as on date to manage *Mtb*. Phytoconstituents like imperatorin, isothiocynate, nucleocidin, artimisinin have been empirically reported for the management of MDR-TB infections. [14- 20] Systematic *in silico* models to select such herbals or phytoconstituents are not available. We have earlier shown successful utility of an *in silico* bioprospection model to identify herbal leads against New Delhi metallo-beta-lactamase-1 (NDM-1). [21] The model was found useful in another study carried for selecting promising plants against *Pseudomonas aeruginosa*.[22] In this informatic study, we have utilized an integrated approach of classical literature surge, matrix based priority modeling & decision making, optimization and validating using molecular docking. The present study is an attempt to use this algorithm to bioprospect bioconstituents from selective plant databases. Such phytoconstituents as 'active ligands' using molecular docking are expected to provides significant leads in management of *Mtb*, after proper validation.

MATERIALS AND METHODS

Bioprospection study for the selection of herbals

A seven steps methodology was followed as detailed below:

a. *Identification of bioactivity parameters (BAP)*: A number of significant bioactivity parameters targets were selected from the reported literature involved inhibiton of

five testing parameters (a) Mycolic acid; (b) Mycobactin; (c) Efflux pump; (d) Antigen 85 complex and (e) Sigma factor (fig. 1). [23- 26]

b. *Evaluation of relevance factor of bioactivity parameters/ physiological targets by using scoring matrix approach*: The study was conducted by using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) as a search tool, working on the principle of Academic Search Engine Optimization (ASEO). *Advanced search model* in which the combination keywords as 'Bioactivity parameters' + 'Antimicrobial activity' were used and yielded 'N' number of hits. The first n=20 hits provided by the advance search were subjected to individual interpretation of relevance factor as a prioritized sample set. On the basis of coefficient of association, the relationship among variables as an intuitive and empirical data, have been developed as % relevance (average). This bioprospection analysis was used to evaluate the net weightage of each bioactivity parameter, using the following formula:

$$\% \text{ Relevance} = \frac{\text{Relevant Hits}}{20} \times 100$$

c. *Classical Bioprospection to select potential herbal candidates for analysis:* Classical Bioprospection model was used to select various plants identified on the basis of extensive literature surge using three descriptors. [27 – 76] The descriptors include: (a) Pharmacological activities reported in literature; (b) Use in various traditional medicine system; (c) Direct/Indirect antimycobacterial activity.

d. *Identification of potent herbals using binary coefficient matrix based analysis for each bioactivity parameter:* The working principle of this step is based on coefficient of association, as the study is based on multivariate data and is polythetic in concept. In this order, individual parameters are considered as the variables and their correlation with the selected plants is presented in the form of binary (presence-absence) data. [77] For the selection of suitable plants the score varies between 0 to 5, while the cut off value is the median of 0 - 5. On the basis of median value the plants having more than 03 parameters, reported in PubMed search engine (n= first 20 hits) against 'Bioactivity Parameter + Selected Plant' random search model, were selected. It works as a foundation for the next step of analysis for only those respective plants with ≥03 parameters.

e. *Weightage matrix based analysis to identify net relevance of plants against multiple bioactivity parameters*: This step filters those plants which are potent with highly weighted score. It involves evaluation of overall weightage of plants (Scores > 3 in previous step) by multiplying their binary score with weightage. This step identifies potential plant leads based on *in silico* bioprospection approach.

f. *Fuzzy set analysis*: The working principle of this step is fuzzy logic or many - valued logic. These values were determined by using the formula given below, in order to make the score of selected plants in a range between 0 to 1. The plants with the obtained value 1 are considered to be surely effective while those with value 0 are neglected or considered as negative control and plants with value lesser than 1 but greater than 0 are considered to be partly effective.

$$\mu s = \frac{[(S) - \min(S)]}{\max(S) - \min(S)}$$

where, µS is the Fuzzy value; S is the Weightage matrix score.

g. *Optimization of data using decision matrix score analysis*: In this approach the numerical value of scores obtained was converted into a leveled score by using a scaled magnitude represented by a

The data based analysis was conducted thrice to assess time based variation in a stipulated period. The significance of tested variables was evaluated by using comparing mean values. Confidence level chosen for the study is $p< 0.05$.

Molecular Docking

The receptor-ligand interaction is the primary mechanism in the drug designing. Hence the native structures of the receptors were used as drug target in order to determine the E-value and compared it with the standard (drug).

a. *Identification of physiological targets:* On the basis of intensive literature perusal the most relevant metabolically important physiological factor were selected in relation to each bioactivity parameter as potential target for the molecular docking study. These include: (a) AccD5 of Acyl co- A carboxylase, (b) Salicylate synthase, (c) Antigen 85, (d) Transcription regulator of Mmr efflux pump and (e) RNA Polymerase- binding Protein A (Rbp A).

b. *Ligand selection:* The phytoconstiutents from the selected plants and standard chemotherapeutic agents like Isoniazid, Isochorismate, Ebselene, Erythromycin, Sorangicin respectively for each physiological target were selected on the basis of literature surge.

c. *Procuration of target protein and Prediction of active site*: The receptor-ligand interaction is the primary mechanism in the drug designing. Hence, the native structure of the receptor is used as drug target. The crystal structure of each physiological target was retrieved from Molecular modeling database (MMDB). Identification of ligand binding site/active site/ pocket of receptor is an important step for drug designing and molecular docking. Larger the pocket shaped region on the protein surface better will be the binding of ligand with the receptor and to determine the pocket on the receptor protein DoG Site Scorer, a web based server was used for the binding site prediction, analysis and druggability estimation. DoG Site Scorer deduces the druggability score of the target protein by elucidating the volume, suface, lipo surface and depth of the individual pocket present on the target protein.

d. *Attainment of ligand:* The crystal structures of all phytoligands and standard chemotherapeutic agents were retrieved from pubchem in the form of 3D Structure Data File (SDF) further the molecules were converted into smi-SMILES format by using OpenBabel Graphical user interface program (http://openbabel.org/docs/dev/GUI/GUI.html). The pdb format of all ligands was attained by online SMILES translator and structure generator (http://cactus.nci.nih.gov/translate/).

e. *Molecular Docking:* Molecular simulation was performed by using Hex 6.12 which involves the shape complimentarity, 3D- Fast Fourier Transform (FFT) mode, 0.6 grid dimension and 180° range angle of receptor and ligand to calculate the linear relationship based binding energy of receptor-ligand complex.

RESULTS

The order of precedence of bioactivity parameters (BAP) on the basis of % relevance and calculated weightage score is: Mycolic acid > Mycobactin > Efflux pump > Antigen 85 > Sigma factor, as depicted in fig. 2.

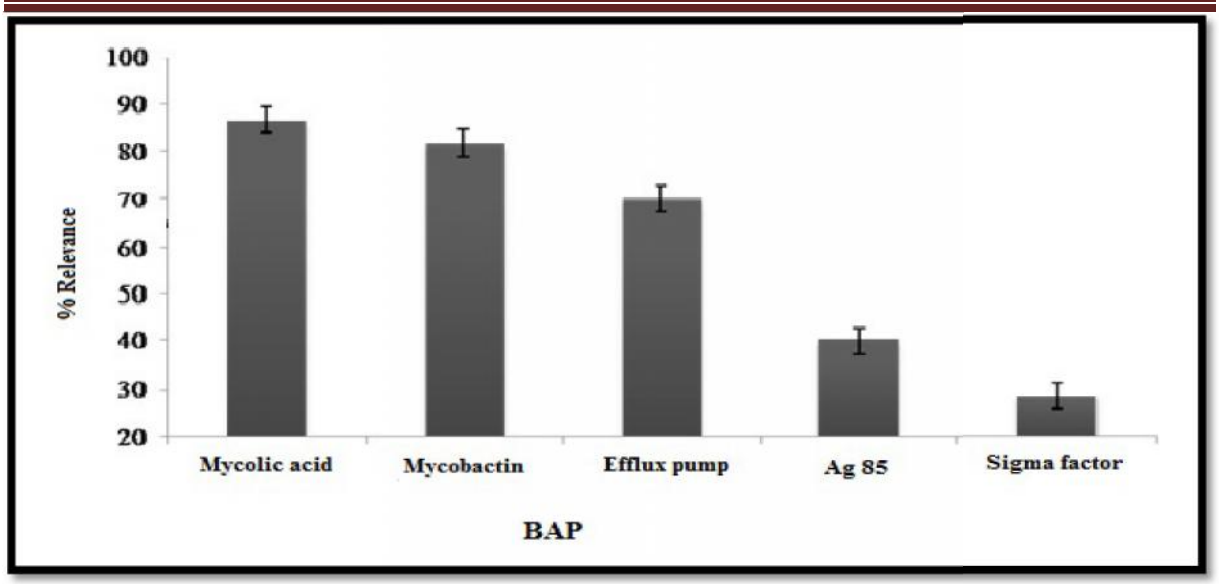

Figure 2: Percentage Relevance of Identified Bioactivity Parameters (BAP) using Scoring Matrix based analysis

The significance of each BAP in virulence is described in Table 1.

Table 1: Rationale for the selection of Bioactivity parameters

S. No.	Bioactivity Parameter	Role
1.	Mycolic acid	i) The waxy, hydrophobic cell wall of *Mtb* is made up of Mycolic acid which are mainly, α- branched saturated fatty acids with 80-Carbon chain length ii) It creates a lipid shield which inhibits entry of cationic proteins, acids, lysozyme, detergents, oxygen radicals of phagosomes and antibiotics iii) Ultimately cell wall helps in building up the resistance towards Isoniazid, Rifampicin, Pyrazinamide and Ethambutanol
2.	Mycobactin (Siderophore)	i) Siderophore are the molecules responsible for iron acquisition in the host cell ii) *Mtb* exploits two types of such molecules to capture iron from human host i.e., mycobactins and carboxymycobactins iii) They can easily permeate the cell hence they helps the other compounds to get enter into the cell iv) Siderophore are essential for microbial viability as iron is one of the important metal ion hat involves in various metabolic activity like redox reaction
3.	Antigen-85	i) Ag 85 is protein complex involves Ag85A, B and C ii) It contributes in cell wall synthesis by employing their mycolyl transferase activity in order to synthesize Trehalose-di-mycolic, an envelope lipid and arabinogalactan-mycolic acid of cell wall
4.	Efflux-pump	i) Efflux pumps (EPs) are the transporters that help in im-

		parting resistance towards various antibiotics in *Mtb* ii) In *Mtb* main EPs belongs to the family ATP-binding cassette (ABC); major facilitator super-family (MFS); small multidrug resistance (SMR) and resistance nodulation division (RND) ii) A novel EP from ABC family identified to confers resistance to β- lactam antibiotics iii) Tap and LfrA from the family MFS presenting resistance towards tetracycline and fluoroquinolones respectively iv) Mmr confers resistance to acrifalvine, ethidium bromide and erythromycin, belonging to SMR family v) The product of mmpL7 gene from RND transporter showing resistance to Isoniazid
5.	Sigma factor	i) Transcription factor, instigate the expression of different regulons ii) 13 sigma factors are present that are involved in expression of different genes iii) For ex.- Expression of Heat shock respond genes are regulated by σB, σE and σH

As shown in Table 2, 30 herbals were identified on the basis of their comparative ethnopharmacological importance, relevance of herb in traditional medicine and their direct and indirect anti-mycobacterial activity reported.

Table 2: Preferred Herbals and their related importance

S.No.	Plants	Pharmacological activities reported in literature	Use in various traditional medicine system	Direct/Indirect antimycobacterial activity
1.	*Allium sativum*	• Cloves have been used for antibacterial, antimycotic, bronchitis, constipation, joint pain and fever • Allicin, the active compound is antimicrobial, lipid reducing, anti oxidative and fibrinolytic	Ancient Chinese to Egyptian medicinal system	Active against MDR strains
2.	*Angelica sinensis*	• Potent bioactivities including antimicrobial, anticancer, anti-inflammatory, hepatoprotective, nephroprotective, protective	Chinese traditional medicinal system	Imperatorin a major constituent reported to be synergistic with first-line antituberculosis drug

		against chronic bronchitis, cold, flu, cough, fever, arthritis etc. • Dietary supplement blood tonic and to cure gyneacological ailments		
3.	*Arracacia tolusensis*	• Antibacterial, anti-inflammatory and anti-hyperglycemic effect • Coumarins and essential oils are reported to have activity against Gram positive and negative bacteria	Ancient Chinese	Isoimperatorin, osthol, suberosin, 8-methoxypsoralen (8-MOP) etc., shows antimycobacterial efficacy
4.	*Artimisia annua*	• Effective against cold, cough, diarrhea also showing antimalarial, antipyretic, antispasmodic, anti-HIV activity • Sesquiterpene lacton is repoted to have antimalarial and anti-HIV activity	Chinese medicinal system	Artimisinin is reported to be effective against Plasmodial malaria, *Mycobacterium tuberculosis* and HIV
5.	*Artimisia nilagirica*	• Significant larvicidal activity (*Aedes aegypti*). Antifungal(*Colletotrichum sp.*), Antibacterial(*Staphylococus aureus* & *Pseudomonas aeruginosa*) and Insecticidal against (*Spodoptera litura*) • Essential oils containing mojorily camphor, camphene, α-thujone, 1,8- cineole, ϒ-muurolene and β- caryophylene showing significant efficacy larva of dengue vector,	Ayurveda and Chinese traditional medicine system	Synergistically active with first-line antimycobacterial drugs

		Aedes aegypti		
6.	*Asclepia fruiticosa*	• Purgative in nature and useful to treat intestinal troubles like stomachache. Singnificantly active against nasal-discharge, cough, Rheumatic pain and anthelmintic especially for *Ascaris lumbricoides* • Phytol, a diterpene is contributing in the anticancerous, anti-inflammatory and antimicrobial activity	South African Traditional medicine system	Active synergism with first-line animycobacterial drug
7.	*Berchimia discolor*	• Effective against Menorrhagia, skin itching and nose bleeding • Prenylated flavanoid , nitidulin, amorphigenin and dabinol exhibits cytotoxic activity against human's prostate cancer cell at experimental level	South African Traditional medicine system	Acetone extract reported to be active against TB and MDR-TB
8.	*Bridelia micrantha*	• Used to treat stomachache, tapeworms, headache, diarrhea and fever • Methanolic extract reported to be active against β- lactam resistant Gram negative bacilli	South African Traditional medicine system	n-Hexane fraction of the extract containing Benzene, 1,3- bis(3-phenoxyphenoxy), 2-pinen-4-one,N(b)-benzyl-14-(carboxymethyl) and linalool exhibits antimycobacterial activity against non-resistant and first-line drug resistant *Mtb* strains
9.	*Carica papaya*	• Immunostimulant, antifertility drug and to treat gastric problem, fever, asthma, dysentery, wounds and burns • Latex and root, seeds	Ayurveda and Chinese medicinal	Against first line and second line drug resistant strain of *Mtb*

		extract and pulp inhibits *Candida albicans, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis etc.*		
10.	*Cimifugae rhizome*	• Anti-inflammatory, antitussive, sedative and also reported to cure cough, muscular rheumatism, rheumatoid arthritis and tinnitus • Effective to treat oligospermia	Chinese medicinal system and South African medicinal system	Against first line and second line drug resistant strain of *Mtb*
11.	*Citrus aurantifolia*	• Effective against diarrhea, dysentery, sore throat, oral thrush, fever including influenza, malaria and jaundice also showing Spasmolytic and antimicrobial effect • Anti-cancer activity	Ayurveda	Inhibits Isoniazid resistant *Mtb* strain
12.	*Citrus sinensis*	• Showing positive inonotropic effect, antifungal, anti-bacterial, antiperoxidation, hypoglycemic and insulin stimulatory activity • Extract (containing coumarin, flavanoid and sterols) have shown anti-osteoporotic activity	Ayurveda, Unani and Chinese	Active compound Decanal, caryophyllene oxide, and palmitic acid from Hexane extract are anti-*Mtb* in nature
13.	*Cnidium monnieri*	• Anti-fungal, anti-bacterial, anti-viral, anti-tumor, anti-oxidation, anti-inflammation activity	Traditional Chinese medicine	Coumarin like imperatorin are present that are reported to shown anti-tubercular activity
14.	*Duroia macrophylla*	• Active compounds like oleanolic and ursolic acid isolated from dichloromethane extract showing anti-	Traditional Chinese medicine	-

		tuberculosis activity against Isoniazid resistant, Rifampin resistant and non-resistant strain of *Mtb*		
15.	*Ficus cordata*	• Active compounds like β- amyrin acetate, lupeol, catechin, epiafzelechin and stigmasterol having hepatoprotective activity • Also reported to be active against Filariasis, diarrhoeal, oral and tuberculosis infection	South African traditional medicine	-
16.	*Foeniculum vulgare*	• Effective in obesity, cardiovascular disorder and hyperlipidemia • Also having antioxidant, hypoglycemic, anti-microbial, hepatoprotective, and memory enhancig property.	Ayurveda, Unani and Chinese medicinal system	Effective against Tuberculosis synergistically with Ist and IInd line drug resistant Tuberculosis
17.	*Gardenia jasminoides*	• Reported to have anti-inflammatory, antipyretic, diuretic, laxative, antihepatitis, birth controlling, anti-artherosclerosis, anti-platelet aggregation, anti-hyperglycemic, anti-hypertension effects. • Also useful to treat gastritis and gastric cancer	Chinese folk medicine and Japanese traditional medicinal system	-
18.	*Glehnia littoralis*	• Anti-mutation and anti-tumor effect due to presence of furanocoumarins • Sedative and analgesic effects	Traditional Korean, Jaanes and Chinese medicine	Showing anti-tubercular activity
19.	*Juniperus communis*	• Anti-diarrhoeal, anti-inflammatory, astrin-	North America tradition-	Isocupressic acid, communic acid and

		gent anti-bacterial and also useful to treat cold, cough and abdominal disorders.	al medicinal system	deoxypodophyllotoxin were identified to be principle antimycobactreial components present in the methanolic extract
20.	*Murraya koenigii*	• Shows Cardiac tonic, antidiabetic and cholesterol reducing property, anti-diarrhoea activity, antioxidant and antimicrobial activity	Ayurveda	-
21.	*Notopterygium incisum*	• Used to treat rheumatoid arthritis, headache, cold	Traditional Chinese medicine	Main constituents are isoimperatorin and notopterol are present
22.	*Olea europea*	• Useful to treat tuberculosis, eczema, cold, stomach disease, kidney pain, malaria, arrhythmia, intestinal pain, as a blood purifier and also it strengthen body muscles	Traditional medicine in Mediterranean areas	-
22.	*Securidaca longepedunculata*	• Effective against arthritic, painful, type 2 diabetes mellitus as it showsanalgesic, anti-inflammatory nad hypoglycemic effects	Traditional South African medicinal system	-
23.	*Solanum panduriforme*	• Effective to treat Pelvic pain, gonorrhoea, wounds, toothache and anti-microbial in nature	Traditional South African medicinal system	-
24.	*Stevia rebaudina*	• Anti-oxidant, anti-diabetic/hypoglycemic, reducing liver and kidney damage	Traditional South America	Nicotinic, alpha-picolinic acid hydrazides and glycosides inhibits the growth of *Mtb* at *in vitro* level
25.	*Terminalia sericea*	• Aqueous and Methanolic extract are repoted to have acivity against *Staphylococcus aureus* and HIV	Traditional South African Medicinal system	Acetone extract have been reported to be active against *Mtb* strain

26.	*Vetveria zizanoides*	• Anti-inflammatory, anti-septic, aphrodisiac, cicastriant, nervine, sedative, healing, calming properties also useful to treat gout, rheumatism, arthritis, muscles aches, dryness, cramps and dry skin	Ayurveda, Unani and traditional Chinese medicine	Hexane fraction of the root extract is reported to have inhibitory activity against *Mtb*
27.	*Warbugia salutaris*	• Respiratory ailments, sinus, rheumatism, backache, stomach ulcers, tooth ache, influenza, malaria, protozoal infection, bacterial infection, dermatological ailments, burns, diarrhea, inflammation of urethra etc.	Traditional African Medicine	A purified drimane sesquiterpenoid lactone, 11a-hydroxycinnamosmolide exhibit antimycobacterial activity
28.	*Ximenia caffra*	• Antibacterial, antifungal, antigonococcal, anti-HIV-type 1 reverse transcriptase	South African Medicinal system	Synergism with First line of drung
29.	*Zingiber officinale*	• Anti-inflammatory, antiemetic and chemoprotective agent • Effective against digestive disorder and also used as appetizer	Ayurveda	Effective against various RTI ailments
30.	*Piper nigrum*	• Anti-inflammatory, Aromatic, muscle-relaxant, Anti-oxidant, Anti-bacterial	Ayurveda	Containing Piperidine as a constituent to which the drug Thioridazine belons i.e., use to target different physiological mechanism of the pathogen

Binary coefficients matrix based analysis of 30 identified plants revealed that, 06 plants namely, *Vetiveria zizanoides, Solanum panduriforme, Piper nigrum, Gardenia jasminodes, Zingiber officinale* and *Foeniculum vulgare* exhibited activity against 03 – 05 virulent factors as represented in Table 3.

Table 3: Binary Score Matrix of Herbals based on Bioactivity Parameter

S.No.	Plants	Bioactivity Parameter					Binary Score	Weightage Score
		Mycolic acid[5]	Mycobactin [4.62]	Efflux pump[3.88]	Antigen 85[2.22]	Sigma factor[1.57]		
1.	*Allium sativum*	1	0	0	1	0	2	7.22
2.	*Angelica sinensis*	1	0	0	1	0	2	7.22
3.	*Arracacia tolusensis*	1	0	1	0	0	2	8.88
4.	*Artimisia annua*	0	1	0	0	0	1	4.62
5.	*Artimisia nilagirica*	0	1	0	0	0	1	4.62
6.	*Asclepia fruiticosa*	1	0	0	0	0	1	5.00
7.	*Berchimia discolor*	1	0	1	0	0	2	7.08
8.	*Bridelia micrantha*	1	0	0	1	0	2	7.22
9.	*Carica papaya*	1	0	0	0	0	1	5.00
10.	*Cimifugae rhizome*	1	0	0	0	0	1	5.00
11.	*Citrus aurantifolia*	1	0	0	0	0	1	5.00
12.	*Citrus sinensis*	1	0	0	0	0	1	5.00
13.	*Cnidium monnieri*	1	0	0	1	0	2	7.22
14.	*Duroia macrophylla*	0	0	1	1	0	2	6.10
15.	*Ficus cordata*	1	0	0	1	0	2	7.22
16.	***Foeniculum vulgare***	**0**	**1**	**1**	**1**	**0**	**3**	**10.72**
17.	***Gardenia jasminoides***	**0**	**1**	**1**	**0**	**1**	**3**	**10.07**
18.	*Glehnia littoralis*	0	0	0	1	0	1	2.22
19.	*Juniperus communis*	1	0	0	1	0	2	7.22
20.	*Murraya*	1	0	0	0	0	1	5.00

	koenigii							
21.	*Notoptery-gium incisum*	0	0	0	0	0	0	0.00
22.	*Olea europea*	1	0	0	0	0	1	5.00
22.	*Securidaca longepedun-culata*	1	0	0	0	0	1	5.00
23.	***Solanum panduri-forme***	**1**	**1**	**1**	**0**	**0**	**3**	**13.5**
24.	*Stevia rebau-dina*	0	0	1	0	0	1	3.88
25.	*Terminalia sericea*	1	0	0	1	0	2	7.22
26.	***Vetiveria zi-zanoides***	**1**	**1**	**1**	**1**	**0**	**4**	**15.72**
27.	*Warbugia salutaris*	1	0	0	1	0	2	7.22
28.	*Ximenia caf-fra*	1	0	0	0	0	1	5.00
29.	***Zingiber of-ficinale***	**1**	**0**	**1**	**1**	**0**	**3**	**11.10**
30.	***Piper nigrum***	**1**	**0**	**1**	**1**	**1**	**4**	**12.67**

The herbal leads (06) selected in previous step were subjected to weighted matrix score analysis utilizing weighted relevance of each bioactivity under consideration (Table-1). Such analysis revealed 04 potent herbal leads. Fuzzy set matrix based optimization of top 06 herbals utilizing relative priority scale of 0-1 led to exclusion of *Zingiber officinale* and *Foeniculum vulgare* with µS < 0.1 (Table 4).

Table 4: Fuzzy Score Analysis of Herbal Leads

S. No.	Herbal Leads	Weightage Score [S]	µs*	Optimization Score#
1.	*Vetiveria zizanoides*	15.72	1	+++++
2.	*Solanum panduriforme*	13.50	0.556	++++
3.	*Piper nigrum*	12.67	0.390	+++
4.	*Zingiber officinale*	11.10	0.076	-
5.	*Foeniculum vulgare*	10.72	0.011	-
6.	*Gardenia jasminoides*	10.07	0.000	-

*$\mu S = [(S) - min(S)] / max(S) - min(S)]$, where µS is the Fuzzy value and [S] is the Weightage matrix score; Max(S) = 15.72(*Vetiveria zizanoides*); Min (S) = 10.72 (*Foeniculum vulgare*); Herbal Leads with Binary Matrix Score ≥3. # Optimized Score Range- 0.10 – 0.20 = +; 0.20 - 0.30 = ++; 0.30 - 0.40 = +++; 0.40-0.50 = ++++; 0.5- 1.0 = +++++

Detailed pathophysiology leads to the identification of the significant physiological target as described in Table 5.

Table 5: Rationale for the selection of Physiological targets

S. No.	Bioactivity Parameter	Role
1.	Acyl co – A carboxylase (ACC)	i) The cell wall of *Mtb* is made up of Mycolic acid which are mainly long chain fatty acids ii) ACC are the enzymes that catalyzes the first step in the biosynthesis of long chain fatty acids
2.	Salicylate synthase	i) Siderophores are the salicylate derivative molecules responsible for iron acquisition in the host cell ii) Salicylate synthase are the first enzymes involves in the biosynthesis of these molecules
3.	Antigen-85	i) Ag 85 is protein complex involves Ag85A, B and C ii) It contributes in cell wall synthesis by employing their mycolyl transferase activity in order to synthesise Trehalose-di-mycolic, an envelope lipid and arabinogalactan-mycolic acid of cell wall
4.	RNA Polymerase binding protein A (RbpA)	i) RbpA is essential for the growth and survival of *Mtb* ii) In *Mtb* it binds to the β- subunit of RNA polymerase and also actively binds in the presence of rifampin iii) It also interacts with the sigma factor (σ^A and σ^B) and thereby promotes initiation complex formation
5.	Rv3066 Transcription regulator	i) Rv3066 are the transcription regulator, instigate the expression of regulons of Mmr efflux pump ii) Mmr are the efflux pumps that exhibits inherent resistance towards EtBr and erythromycin while over expressed in the presence of isoniazid

The tertiary structure with possible number of pockets is developed by using MMDB and Dog SiteScorer online tool (Fig 3, 4, 5, 6, 7). The analysis of phytoconstituents from 06 herbals revealed 18 constituents to be chosen for the docking study (03 from each plant). The selected phytoconstituents were subjected to molecular docking in order to reveal interaction energy.

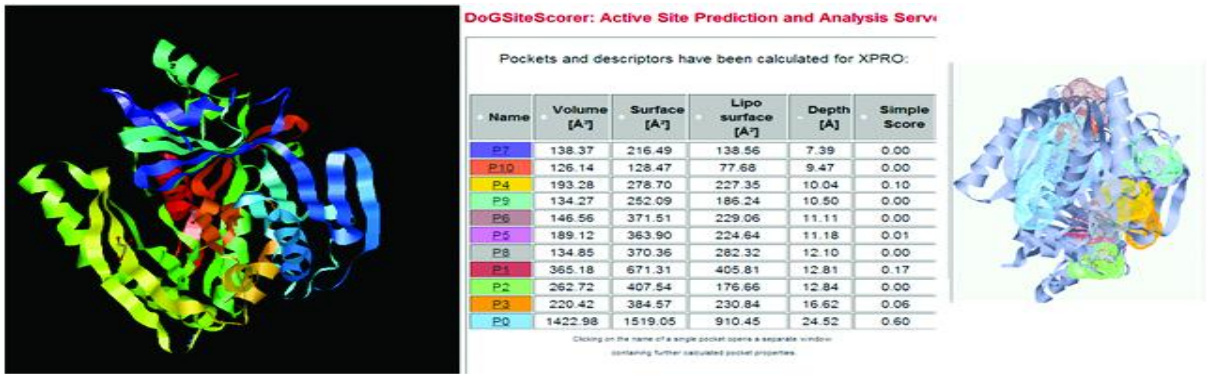

Figure 3: The crystal structure and pockets of Acyl Co-A carboxylase domain 5

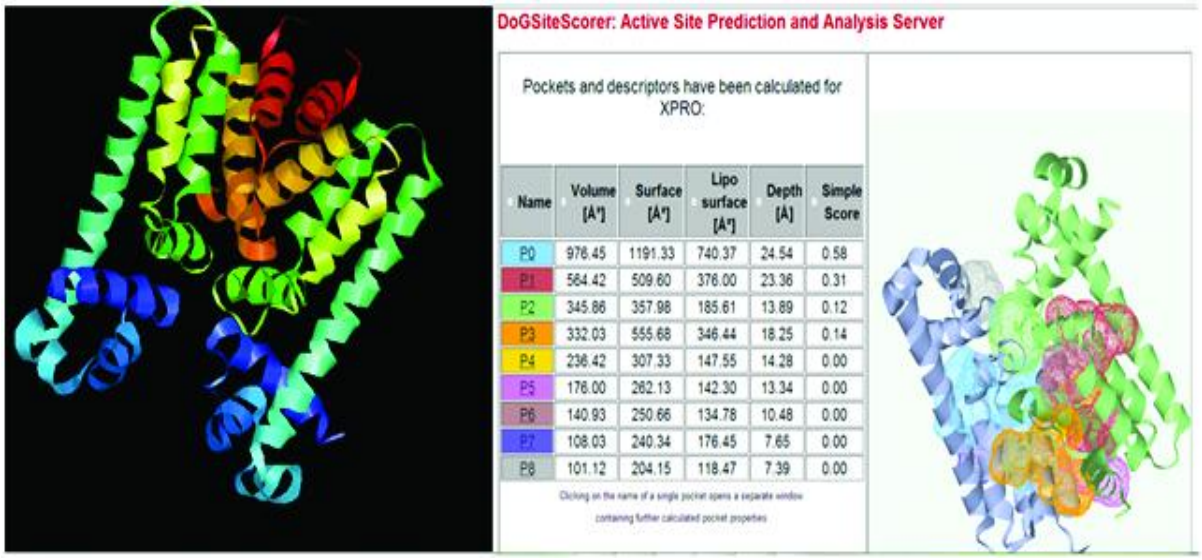

Figure 4: The crystal structure and pockets of Antigen 85

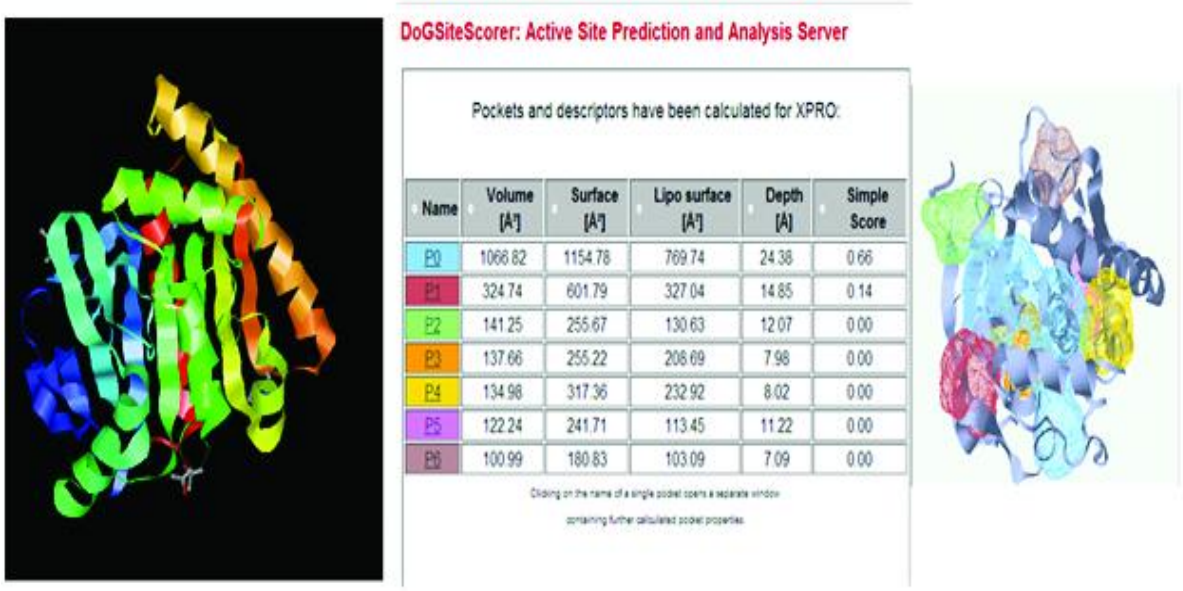

Figure 5: The crystal structure and pockets of Rv3066 Transcriptional regulator of Mmr Efflux pump

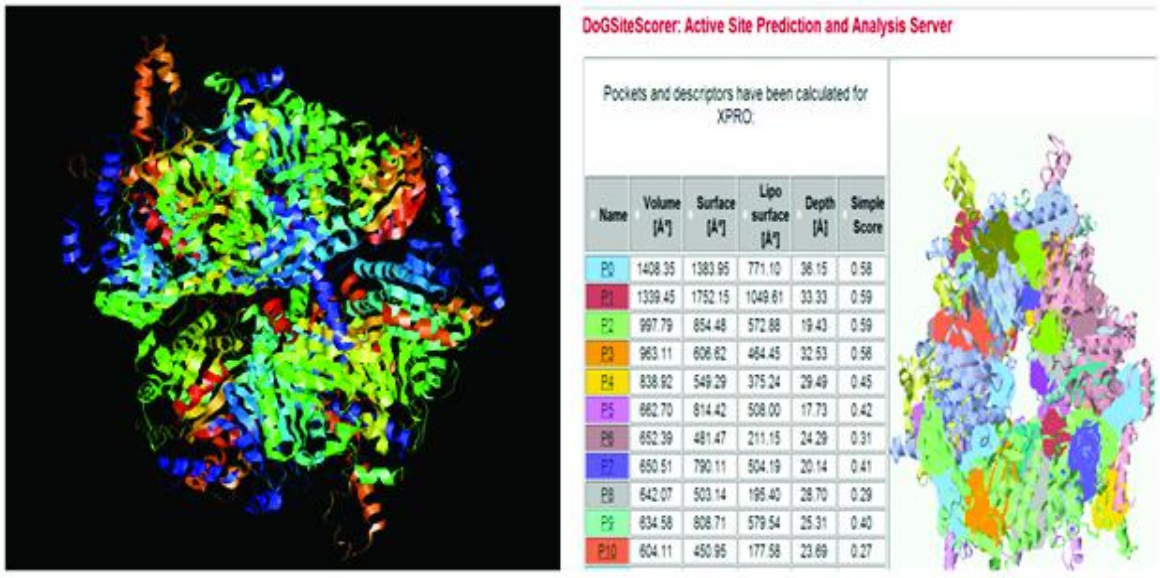

Figure 6: The crystal structure and pockets of Salicylate synthase

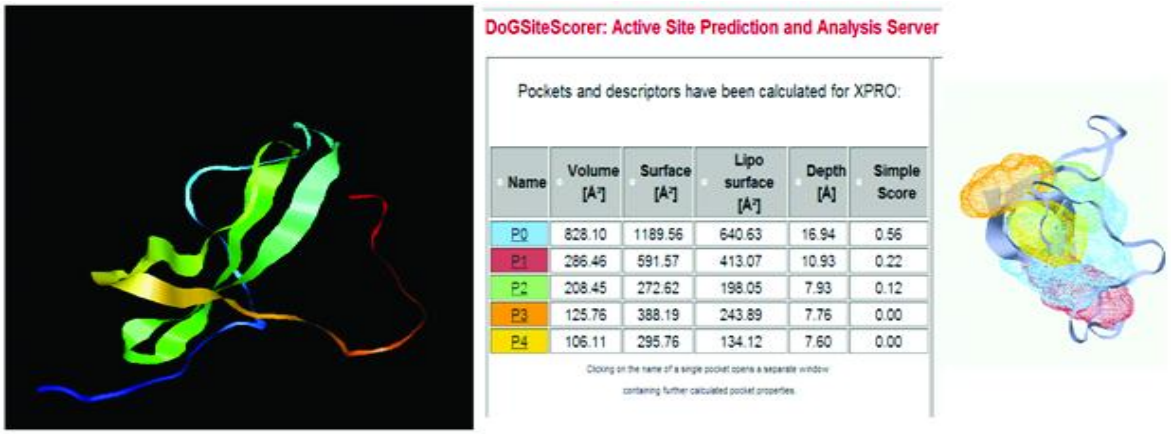

Figure 7: The crystal structure and pockets of RNA polymerase binding protein

On the basis of relative study it was revealed that the phytoligands have shown significantly considerable binding energy with individual physiological target when compared with the standard chemotherapeutic agents, as depicted in Table 6.

Table 6: E- value of Preferred Phytoconstituents from selected plants as compared to standard

Physiological target	E-value of phytoligands from selected plants																	Standard with E-value
	Vetiveria zizaza-noides		*Solanum panduriforme*			*Piper nigrum*			*Gardenia jasminoides*			*Zingiber officinale*			*Foeniculum vulgare*			
	Vetivone	**Khusimol**	**Zizaenen**	**Sapogenin**	**Salsodine**	**Yamogenin**	**α Caryophyllene**	**Piperenene**	**Carene**	**Gardenoside**	**Geniposide**	**Gingerol**	**Paradol**	**Zingiberene**	**α Phallendrene**	**Estragole**	**α Anetholle**	
AcD5	-228.21	-166.60	-166.60	-290.69	-312.93	-312.93	-187.15	-291.03	-184.50	-278.84	-213.21	-286.51	-300.57	-301.57	-216.35	-175.26	-192.91	-196.50	**Isoniazid: -163.80**
Ag85	-118.12	-101.51	-103.16	-168.26	-174.92	-172.27	-186.64	-290.84	-164.61	-163.65	-123.27	-167.10	-114.42	-193.98	-125.95	-96.70	-111.93	-114.42	**Ebselen: -149.08**
Salicylate synthase	-203.32	-2144.34	-204.27	-336.32	-273.32	-288.13	-203.38	-269.62	-160.21	-310.96	-2006.62	-300.64	-302.99	-274.87	-229.87	-170.06	-184.03	-189.82	**Isochorismate: -213.67**

RbpA	-148.45	-138.89	-138.89	-199.93	-201.41	-203.36	-145.32	-193.58	-115.37	-203.44	-157.52	-213.19	-318.67	-323.73	-160.48	-126.30	-134.70	-137.99	**Soranginicin: -316.18**
Rv3066	-189.67	-177.07	-163.38	-277.90	-258.04	-244.83	-179.48	-249.20	-143.09	-203.44	-157.52	-213.19	-311.35	-299.94	-198.76	-148.01	-170.60	-178.25	**Erythromycin:-298.47**

DISCUSSION

The history of antibiotics discovery and drug resistance build-up in microbes goes hand in hand as one lead to the progressive development of the other. Emanation of drug resistant strains necessitates rapid investigation of effective antimicrobial modalities [22, 33-35] while on the other hand, the ethnopharmacological importance of a number of medicinal plants and its proper implementation as in medicine is still a matter of deliberation. This study is a presentation of classical bioprospection in a rhetoric way by using holistic mathematical approach to obtain unbiased results, validated by molecular docking. The process involves targeting bioactivity parameters by literature survey, their precedence indexing, and score assessment preparation of large database of plants and on the basis of scoring, decision matrix and optimization of their final weightage. The present study demonstrated in silico approach to target numerous bioactivities like Mycolic acid and Efflux pump of *Mtb*, responsible for contribution towards emergence of multi-drug resistance. Mycolic acid creates a lipid shield which hinders the entry of cationic proteins, acids, lysozyme, detergents, oxygen radicals of phagosomes and antibiotics like Isoniazid, Rifampicin, pyrazinamide and ethambutanol. Efflux pumps (EPs) are the transporters that help in imparting resistance towards various antibiotics in *Mtb*.

One of the major process of inhibition of resistance offered by *M. tuberculosis* is Mycolic acid formation is essential to confines the diffusion of antibiotics. Mycolic acid, a protective shielding material of cell wall encapsulating the micro-organism, prevents penetration of antibiotics. Isoimperatorin, osthol, suberosin, 8-methoxypsoralen (8-MOP), various essential oil etc. from *Angelica sinensis, Aracacia tolusensis, Cnidium monnieri, Citrus aurantifolia* were reported to interrupt Mycolic acid formation pathway as acting synergistically with the Isoniazid and other first- line defense drugs aginst *Mtb.* Tricyclic sesquiterpenes- khusenic acid and khusimol(*Vetiveria zizanoides*); essential oils - gingerol (*Zingiber officinale*); piperene (*Piper nigrum*) (blocks the efflux system and synergistic to EtBr); linoleic acid, oleic acid , 1,3- benzenediol, undecanal and 2,4- decadienal (*Foeniculum vulgare*); isoimperatorin(*Gardenia jasmanoides* and *Solanum panduriforme*) were reported to inhibit the growth of *Mtb* variously also act

synergistically with different first line drugs those which are directly affect Mycolic acid formation. These identified indicators can be utilized to screen 'drug like molecules' from these prioritized herbal leads.

Herbal leads were selected on the basis of binary matrix (i.e., present or absence of the bioactivity) is in line with work reported by Cheetham and coworkers (1969) that made use of binary coefficients for interpretation of multivariate bioassociational data. The fuzzy score based optimization revealed that *Vetiveria zizanoides; Solanum panduriforme; Piper nigrum; Zingiber officinale; Foeniculum vulgare* and; *Gardenia jasanoides* are potent herbal leads to be tried against *Mtb*.

M. tuberculosis acyl- CoA carboxylase (Acc) is the enzyme that is the mainstay for the synthesis of long chain fatty-acid molecule of the Mycolic acid of the cell wall. This enzyme is made up of six domains (Acc D 1-6) out of them AccD5 domain is the main essential part at where the synthesis of methylmalonyl- CoA initiates, that is the foundation of the multimethyl- branched fatty acids of the cell envelop.[78] Salicylate synthase catalyzes the first committed step in the biosynthesis of Siderophore (mycobactin).[79] Ag 85, a major secretion product of *Mtb* also immunogenic in nature. [80] Rv3066 are the transcription regulator, instigate the expression of regulons of Mmr efflux pump. RbpA is essential for the growth and survival of *Mtb*. In *Mtb* it binds to the β- subunit of RNA polymerase and also actively binds in the presence of rifampin.

CONCLUSION

Our analyses have demonstrated matrix (Weightage, Binary and Fuzzy) based herbal informatics approach, is a fast, reliable and systematic model to target multiple pathophysiological foci altered during onset and spread of disease. Such model works on the principle of multivariate analysis of bioassociational data with segregation and filtration at each stage. This study has provided 06 herbal leads against *Mtb,* which, in turn, needs to be evaluated further at *in vitro* and *invivo* level.

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