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In-silico Analysis, Homology Modelling And Docking Studies Of Essential Proteins Of *Mycobacterium Leprae* For Effective Treatment Of Leprosy.

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ABSTRACT

The high emergence of multi-drug resistant strains of bacteria like *Mycobacterium leprae* caused the existing drugs to be ineffective against them. This impacted a need to quest novel targets and drug compounds to treat diseases like leprosy. Protein sequences of *M.Leprae* which are non-homologous to humans, participate in essential metabolic pathways of the bacteria and are necessary for the pathogen to survive were taken for study. Physiochemical characterisation, structural and functional analysis were carried out on these proteins. Their 3D structures were predicted were evaluated using various servers and workspaces. It was found that the proteins under study are acidic, thermostable and cytoplasmic in nature. Docking studies revealed that the herbal compounds which have least or no side effects were much more efficient than the chemical drugs. LysR family transcriptional regulator and MurE proteins of *M.leprae* were found to be the best targets to make novel drug formulations against the bacteria. Hops extract from *Humuluslupulus*, and Daucosterol from *Justiciaadhatoda* have maximum binding energies with the proteins under study. Thus the study showed that the herbal compounds interacted better with the proteins than the market drugs and were subjected to experimental evaluation to test their efficiency to treat leprosy.

Keywords: Homology modelling, Molecular Docking, Leprosy, Mycobacterium Leprae, Herbal compounds, Drug discovery.

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INTRODUCTION

The unusual increase in population and drastic misuse of resources lead to heavy pollution which poses a significant threat to humans. The alarming effect of this pollution is that most of the pathogens already became resistant to more than one drug *Mycobacterium leprae* is one amongst them. UNIPROT defines *Mycobacterium leprae* as uncultivable, gram positive, nonmotile, acid-fast, rod-shaped, obligate intracellular bacterium[1]. The Norwegian physician Gerhard Armauer Hansen discovered it in 1873 [2]. Because of its slow replication rate and being an obligate intracellular parasite, it is much difficult to grow it in a laboratory [3]. The organism was thought to have originated in East Africa and spread across the world through migration of humans [4]. After entering a Human body, they can grow within the macrophages to evade the immune system and express many virulence factors that are essential for nutrient access and invade nerve cells [5]. Fortunately the 1st genome sequence of a strain of *M.Leprae* was done in 1998 [6]. This bacteria is the causative organism for a dreadful disease called leprosy or Hansen's disease. Leprosy is an infectious disease that causes nerve damage, skin sores and disfiguring in arms, legs, etc. This disease has been present since ages affecting some thousands of people, often surrounded by horrifying, negative stigmas and tales of affected people being ostracized. The disease panicked almost people of every continent. But leprosy is not very contagious as one can catch it if present in proximity and repeated contact with mouth and nose droplets with the patient. Children are more likely to affect than adults. It takes about 3 to 5 years for the symptoms to appear after the bacteria enters the body [7]. According to World Health Organization official report from five WHO regions, the globally registered number of leprosy cases in the year of 2013 was 180,618. In 2016 WHO launched the "Global Leprosy Strategy 2016–2020: Accelerating towards a leprosy-free world" [8] to reinvigorate efforts to control and avoid disabilities caused by leprosy, especially among children who are prone to get affected in endemic countries [9]. The first encounter of the disease occurred in the 1940s followed by the development of the drug dapsone. But later in the 1960s, the bacteria became resistant to dapsone, the only anti-leprosy drug of that time. So rifampicin and clofazimine were discovered and given as a treatment for the disease, thus named it as multi-drug therapy.

The resistant strains have been reported since 1964 [10-12]. The resistance is due to mutational changes in some of its genes like gyrA, rpoB, and folP which developed resistance to drugs like quinolones, rifampicin, and dapsone [13]. The prevalence rate of the disease has dropped from 21.1 cases per 10000 people in 1983 to 0.2 cases per 10000 people in 2015. Even though there is an enormous success in curing leprosy around the world, the stigma associated with it persists and there is no much awareness among people about the disease which may lead to persistence of the disease. The multi-drug therapy given to the patient has many side effects associated with it. So scientists are still in search of new drugs that can cure the disease. Medicinal plants have been used to treat diseases since ages. These plants may act as potential candidates to develop novel medications to combat multi-drug resistant *M.Leprae*. Great physicists created many formulations which are still unnoticed to treat dreadful diseases. Many plants have been reported antibacterial activity across the globe and were also described in Ayurveda. These should be studied to discover novel treatment methods to cure leprosy. Computational approaches for drug designing and prediction have made a drastic change in treating a disease. Discovering a novel drug in its early stages is not only difficult but also laborious and expensive. This situation made researchers come up with alternative and inexpensive approaches for proposing new drugs for deadly disease causing organisms like *M.Leprae*. The best way to do this is to find drug targets for the pathogens, mainly for the resistant ones. Literature search showed that there were a few reports available which described the computational identification of drug targets in *M.Leprae* [14-19]. Essentiality of genes and its products for the organisms has to be found and are considered as potential drug targets to design antibiotics. The conventional way of finding these genes is too expensive and time intensive. Already there are genomes of around 1000 pathogenic bacteria made available in National Center for Biotechnology Information (NCBI) genome database [20]. So this vast available genomic information when dealt with an efficient computational algorithm, will be productive. Recent advances in bioinformatics have enabled the identification and characterization of possible protein target sites more rapidly than the conventional methods. Even advances in proteomics resulted in functionally predict potential drug targets for these proteins. Further, these two concepts lead to a new approach for identification of novel drug targets by using insilico bioinformatic tools have revolutionized the process of drug discovery [21]. A methodology called genomic subtraction analysis in which the genome of a pathogen is screened against various databases to find genes that are non-homologous to humans, essential for the pathogen to survive and also participate in unique pathogen pathways [16]. Various researchers have reported the applications of successful predictions by this approach [22-33]. Through literature search, it was found that some researchers [34,35] have already

reported a few proteins of *M.Leprae* which were identified through Advanced Genomic Subtraction Analysis. These proteins were taken and targeted against drugs and herbal formulations to determine the possible drug having the best fit with the proteins under study. These proposed herbal compounds can be considered to treat the disease without any side effects or with minimum side effects. So a comprehensive, toxicity and safety clinical studies are needed to test their efficiency in curing leprosy.

MATERIALS AND METHODS

Target proteins

The amino acid sequences of the proteins of *M.Leprae* were taken from National Centre for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov). For present study the accession numbers of protein sequences used were WP_010907637.1, WP_010907513.1, WP_012634437.1, WP_010908819.1, WP_010908840.1, WP_010908961.1, WP_010909041.1, P57994, Q7AQ10, P38056, B8ZQQ0, O69557 and O69556.

Table 1- Accession numbers and abbreviations of proteins of *M.Leprae* used in study.

S.no	Protein	Accession number	Abbreviation
1	Exopolyphosphatase	WP_010907637.1	Epp
2	Haloaciddehalogenase	WP_010907513.1	Hdh
3	Bifunctionaldiguanylatecyclase/Phosphodiesterase	WP_012634437.1	Bdc
4	LysR family transcriptional regulator	WP_010908819.1	Lysr
5	SAM-dependent methyltransferase	WP_010908840.1	Sam
6	Dehydratase	WP_010908961.1	Deh
7	Murein biosynthesis integral membrane protein MurJ	WP_010909041.1	MurJ
8	UDP-N-acetylmuramate--L-alanine ligase	P57994	MurC
9	dTDP-4-dehydrorhamnose 3,5-epimerase	Q7AQ10	RmlC
10	Alanine racemase	P38056	Alr
11	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosaminetransferase	B8ZQQ0	MurG
12	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	O69557	MurE
13	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	O69556	MurF

Physiochemical characterization

Using Expasy ProtParam bioinformatic tool [36], the physiochemical properties like the amino acid composition, molecular weight, Extinction coefficient [37], total no of negative and positive residues, Aliphatic index [38], Instability index [39], number of negative residues (-R), number of positive residues (+R), atomic composition, Grand Average Hydropathicity(GRAVY) [40] and estimated half-life [41,42] were computed.

Structural and Functional Analysis

Using Self Optimized Prediction Method with alignment (SOPMA) the secondary structural features of the proteins like Alpha Helix, Beta turns, Random coils and extended strands were computed [43]. To identify the known motifs in the protein sequences, Motif Scan Server [44] was used. The cellular localization of the proteins was determined using CELLO v.2.5 [45]. The no of cysteine residues present in the protein sequences was found using CYS_REC tool [46].

Tertiary Structure Prediction and Evaluation

By using blastp, taking the proteins sequences as query against PDB, the proteins structural database, it was found that the 3D structures of the above proteins of *M.Leprae* were not present. So they were

modelled by homology modelling technique using Swiss-Model Workspace [47] accessible via the Expasy web server. The server was operated in an automated mode in which only the protein sequence is required. Template selection, alignment, and model building were done automatically.

The results include selected templates, sequence identity and estimation of model quality based on QMEAN4 Score and Z-Score [48]. Predicted models were further evaluated using Verify3D [49,50] PROCHECK [51], RAMPAGE [52], PROQ server [53], and ERRAT server [54] The Predicted 3D structures were visualized in RasMol v 2.7.5 [55] visualization program.

Drugs and Natural Compounds

Drugs and natural herbs that are being used to treat leprosy were identified through literature search. Drugs such as minocycline, clarithromycin, clofazimine, dapsone, ofloxacin, rifadin and natural compounds from medicinal plants i.e, Licorice(*Glycyrrhiza glabra*) [56], Aloe vera (*Aloevera L*) [57], Vasaka(*Justicia adhatoda*) [58], Noni(*Morinda citrifolia L*) [59], Onion(*Allium cepa L*) [60], Garlic(*Allium sativum L*) [61], Neem(*Azadirecta Indica*) [62], Acacia [63], *Dalbergia retusia*[64], Chaulmoogra(*Hydnocarpus wightianus)* [65], Psorelea (*Psorelea corylifolia)* [66], *Berberis spp*[67] and Hops *(Humulus lupulus)* [68] were selected as ligands to dock against the proteins of *M.Leprae*. Their chemical structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Then these were converted to PDB format using Openbabel [70]. All these compounds were screened based on Lipinski Rule of Five [71].

Table 2- Selected natural compounds from various plants using Lipinski Rule of Five

S no	Plant name	Compound	Pubchem ID
1	*Humulus lupulus*	Hops Extract	6850842
2	*Berberis spp*	Berberine	2353
3	*Psorelea corylifolia*	Psorelen	6199
4	*Hydnocarpus wightianus*	Hydnocarpic acid	110680
5	*Dalbergia retusia*	Retusin	5481240
6	*Acacia*	Isoqurcitin	5280804
7	*Acacia*	Spireoside	5320844
8	*Azadirecta Indica*	Salannin	6437066
9	*Allium sativum L*	Allicin	65036
10	*Allium cepa L*	Allin	87310
11	*Morindacitrifolia L*	Glucoronic acid	92283
12	*Justicia adhatoda*	Daucosterol	5742590
13	*Justicia adhatoda*	Vitexin	5280441
14	*Justicia adhatoda*	Vasicine	667496
15	*Aloevera L*	Aloin	12305761
16	*Aloevera L*	Emodin	3220
17	*Aloevera L*	Barbaloin	12305761
18	*Glycyrrhiza glabra*	Glabrol	480768
19	*Glycyrrhiza glabra*	Glicophenone	10021298
20	*Glycyrrhiza glabra*	Licoricidine	480865

Table 3- Selected FDA approved drugs used for docking studies

S no	Chemical compound	PubChem ID
1	Minocycline	5467543
2	Clarithromycin	84029
3	Clofazimine	2794
4	Dapsone	2955
5	Ofloxacin	4583
6	Rifadin	5381226

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Molecular Docking

Hex 8.0.0 cuda [72] was used to analyse the interactions between the drug and the proteins. In the docking studies, proteins were taken as receptors and chemical drugs, natural compounds were taken as ligands both in PDB format.
Based on their binding energy, vanderwal interactions and electrostatic interactions post-screening analysis was carried out.

RESULTS

Physiochemical Characterisation

The Physiochemical characteristics of the proteins that were computed using protparam tool were given in Table 4. The computed pI of all the proteins was <11. The EC values for the proteins were in the range of 3105-97415 M^{-1} cm^{-1}. The instability index says that 3 out of 13 proteins were unstable. The GRAVY index of most of the proteins was positive.

Table 4- Physiochemical characteristics of proteins of *M.Leprae*

Protein	Sequence length	Molecular weight	pI	Extension coefficient	Instabiliy index	Aliphatic index	GRAVY	-R	+R
Epp	317	33489.5	5.01	15720	22.12(stable)	110.44	0.317	42	31
Hdh	281	29291.26	4.88	28085	30.25(stable)	97.62	0.117	34	22
Bdc	623	67463.13	4.95	41160	35.72(stable)	109.57	0.144	88	54
Lysr	174	17430.96	5.12	3105	34.77(stable)	108.74	0.423	16	12
Sam	420	47091.42	6.44	80580	42.07(unstable)	87.6	-0.315	51	47
Deh	300	32906.86	9.61	22920	46.20(unstable)	96.17	-0.019	21	27
MurJ	1206	126962.99	8.54	97415	41.30(unstable)	110.34	0.334	81	84
MurC	495	51588.17	6.4	17920	32.47(stable)	104.83	0.266	47	43
RmlC	202	22126.84	5.09	33710	25.62(stable)	84.5	-0.081	23	15
Alr	388	41083.03	5.61	31525	26.69(stable)	104.36	0.130	43	35
MurG	407	42422.88	10.18	18910	30.08(stable)	104.55	0.168	35	46
MurE	530	54808.46	5.37	21680	28.57(stable)	100.74	0.190	60	46
MurF	517	52874.16	5.48	33585	32.05(stable)	103.83	0.209	57	43

Structure and Functional Analysis

Secondary structure elements were calculated using SOPMA tool. The results were given in Table 5. It was found that the protein Exopolyphosphatase had more Alpha helix content (53.84%) whereas least in RmlC (15.35%). The results of the CELLO server indicate that all the proteins were Cytoplasmic in nature. The no of cysteine residues and disulphide bridges were found using CYC_REC tool. The results given in Table 6 showed that MurC protein was having a maximum of 8 cysteine residues, whereas the protein dehydratase has no cysteine residues. It was also found that none of the proteins has probable disulphide bridges. Using Motif Scan server, the motif regions of the proteins were determined (Table 7). All the proteins sequences have an N-myristoylation site with a higher number of times. There was also a phosphorylation site for Casein kinase II

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and Protein Kinase C in all the proteins. 4 out of 13 proteins has an Amidation site and no N-glycosylation site. The scan results also inferred that 3 out of them has a phosphorylation site for Tyrosine kinase.

Table 5- Secondary structure parameters of proteins calculated using SOPMA tool

Protein	Alpha Helix	Extended Stands	Beta turns	Random Coils
Epp	53.84	18.93	8.52	16.72
Hdh	33.81	21	12.10	33.10
Bdc	49.60	17.82	8.51	24.08
Lysr	35.06	20.11	8.62	36.21
Sam	45.95	16.19	10.48	27.38
Deh	29.67	22.33	7.67	40.33
MurJ	37.65	19.07	7.30	35.90
MurC	33.74	25.05	12.73	28.48
RmlC	15.35	33.17	15.84	35.64
Alr	44.85	18.30	11.86	25
MurG	38.82	18.43	8.35	34.40
MurE	30.57	23.96	11.13	34.34
MurF	37.72	18.18	8.90	35.20

Table 6- No of cysteine residues present in protein sequences predicted using CYC_REC tool

Protein	No of cysteine residues
Epp	5
Hdh	2
Bdc	5
Lysr	3
Sam	5
Deh	0
MurJ	3
MurC	8
RmlC	5
Alr	2
MurG	1
MurE	5
MurF	3

Table 7- Predicted motifs of Proteins of *M.Leprae* using MOTIF SCAN Server

Protein	Motif Information	No of Sites	Amino acid residue
Epp	Casein kinase II phosphorylation site.	5	107-110,113-116,183-186,272-275,303-306
	Amidation site.	1	22-25
	N-myristoylation site	7	41-46,114-119,142-147,151-156,215-220,310-315
	Protein kinase C phosphorylation site.	3	13-15,173-175,303-305.

Hdh	Casein kinase II phosphorylation site.	3	24-27,153-156,211-214
	N-glycosylation site.	1	29-32
	Protein kinase C phosphorylation site.	5	4-6,33-35,54-56,87-89,92-94.
	Casein kinase II phosphorylation site.	5	4-7,26-29,312-315,349-352,460-463
	N-glycosylation site.	1	537-540
	N-myristoylation site	9	40-45,302-307,357-362,465-470,498-503,507-512,535-540,562-567,576-581
	Protein kinase C phosphorylation site.	5	326-328,346-348,375-377,487-489,523-525
Lysr	Casein kinase II phosphorylation site.	1	81-84
	N-myristoylation site	2	2-7,77-82
	Protein kinase C phosphorylation site.	1	73-75
Sam	Casein kinase II phosphorylation site.	7	19-22,80-83,171-174,255-258,297-300,351-354,402-405
	N-myristoylation site	11	6-11,23-28,34-39,54-59,68-73,127-132,199-204,218-223,289-294,371-376,379-384
	Protein kinase C phosphorylation site.	8	39-41,82-84,91-93,146-148,297-299,311-313,324-326,351-353
	N-glycosylation site.	1	301-304
	cAMP- and cGMP-dependent protein kinase phosphorylation site.	2	88-91,122-125
RmlC	Casein kinase II phosphorylation site	5	80-83,102-105,149-152,168-171,188-191
	N-myristoylation site	3	56-61,96-101,184-189
	Protein kinase C phosphorylation site.	2	106-108,168-170
	N-glycosylation site.	3	49-52,104-107,127-130
Deh	Casein kinase II phosphorylation site.	4	15-18,53-56,97-100,172-175
	N-myristoylation site	5	72-77,107-112,154-159,233-238,295-300
	Protein kinase C phosphorylation site.	5	15-17,181-183,197-199,255-257,282-284
	N-glycosylation site.	2	63-66,155-158
	Tyrosine kinase phosphorylation site.	1	247-254

MurJ	Casein kinase II phosphorylation site.	15	108-111,212-215,447-450,581-584,643-646,748-751,769-772,781-784,812-815,859-862,870-873,969-972,985-988,1053-1056,1134-1137
	N-myristoylation site	24	68-73,232-237,271-276,287-292,370-375,457-462,467-472,525-530,562-567,596-601,705-710,720-725,735-740,801-806,825-830,844-849,882-887,901-906,944-949,1002-1007,1029-1034,1086-1091,1173-1178,1187-1192
	Protein kinase C phosphorylation site.	7	586-588,611-613,632-634,640-642,777-779,935-937,985-987
	N-glycosylation site.	2	307-310,598-601
MurC	Casein kinase II phosphorylation site.	8	38-41,65-68,87-90,177-180,189-192,211-214,313-316,403-406
	N-myristoylation site	13	17-22,34-39,72-77,141-146,149-154,162-167,200-205,280-285,302-307,338-343,375-380,412-417,423-428
	Protein kinase C phosphorylation site.	1	79-81
Alr	Casein kinase II phosphorylation site.	5	71-74,220-223,243-246,353-356,379-382
	Amidation site.	2	189-192,309-312
	N-myristoylation site	4	139-144,248-253,270-275,295-300
	Protein kinase C phosphorylation site.	8	7-9,112-114,126-128,133-135,254-256,264-266,371-373,379-381
	N-glycosylation site.	1	214-217
	Tyrosine kinase phosphorylation site.	1	266-273
MurG	Casein kinase II phosphorylation site.	2	4-7,301-304
	Amidation site.	1	10-13
	N-myristoylation site	8	35-40,65-70,128-133,167-172,182-187,226-231,257-262,337-342
	Protein kinase C phosphorylation site.	3	4-6,66-68,399-401
	N-glycosylation site.	1	2-5
	cAMP- and cGMP-dependent protein kinase phosphorylation site.	2	12-15,92-95
	Tyrosine kinase phosphorylation site.	1	73-80

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MurE	Casein kinase II phosphorylation site.	3	41-44,181-184,310-313
	Amidation site.	1	412-415
	N-myristoylation site	12	29-34,37-42,73-78,118-123,136-141,153-158,162-167,219-294,289-294,384-389,451-456,494-499
	Protein kinase C phosphorylation site.	4	51-53,129-131,141-143,293-295
	N-glycosylation site.	1	229-232
MurF	Casein kinase II phosphorylation site.	8	21-24,144-147,232-235,291-294,340-343,363-366,369-372,424-427
	N-myristoylation site	9	45-50,94-99,106-111,135-140,196-201,210-215,224-229,334-339,447-452
	Protein kinase C phosphorylation site.	5	140-142,144-146,191-193,362-364,381-383
	cAMP- and cGMP-dependent protein kinase phosphorylation site	1	28-31

Tertiary Structure Prediction and Evaluation

3D structures of the proteins were predicted using Swiss-model. This server evaluates the model quality based on QMEAN4 global score and QMEAN Z-score. The models were taken based on the best QMEAN Score and Z-score. The predicted 3D structures visualized using Hex were given in**Error! Reference source not found.**. These selected models were further evaluated for their quality using Verify3D, PROCHECK, PROQ server, ERRAT sever (Fig 2) and RAMPAGE (Fig 3). The results of evaluation were given in Table 8. These values showed that the models produced were of fine quality.

Table 8- Validation parameters computed for predicted 3D structures of proteins of M.Leprae

Protein	RAMPAGE	Verify 3D(%)	Procheck(%)	ProQ		ERRAT
	RFR(%)			LG score	Max sub	Overall quality factor(%)
Epp	94.2	99.68	89.4	5.53	0.51	95.47
Hdh	95.8	88	91	5.53	0.47	90.31
Bdc	96.4	91.69	95.2	5.03	0.46	93.93
Lysr	93.5	88.74	87.7	6.82	0.70	87.82
Sam	93.4	91.58	88.1	4.96	0.53	96.21
Deh	95.6	90.40	91	5	0.51	93.36
MurJ	98.8	99.60	94.3	5.73	0.50	97.89
MurC	96.2	97.46	92.5	6.37	0.61	94.09
RmlC	97.1	100	92.6	6.09	0.37	96.01
Alr	96.6	98.11	90.8	7.5	0.63	96.11
MurG	93.4	93.11	90.9	7.88	0.62	80.09
MurE	94.8	98.19	90.4	6.59	0.52	96.28
MurF	91.7	92.94	87	6.09	0.49	91.26
RFR-No of residues in favoured region						

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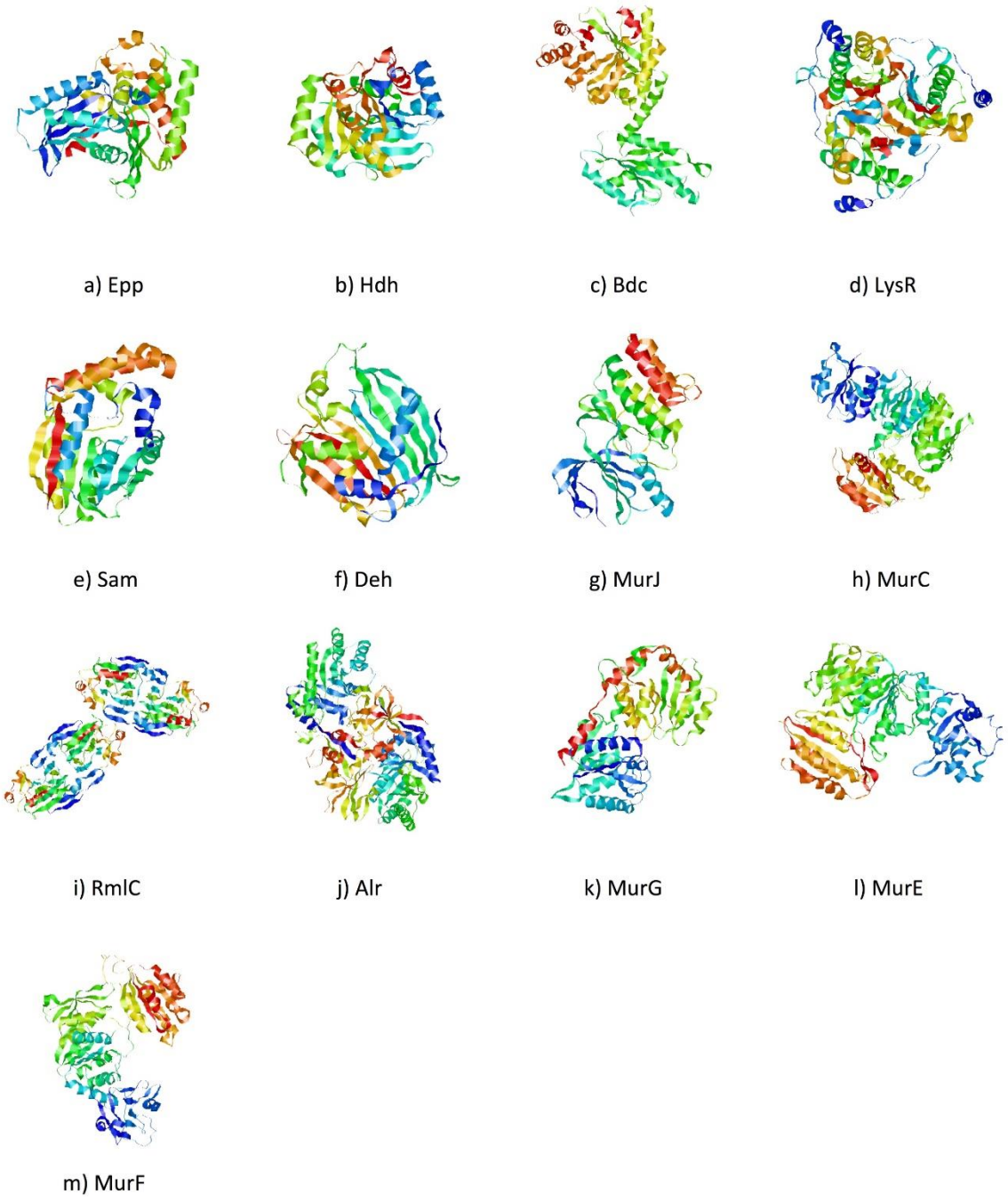

Fig 1- Predicted 3D structures of proteins of *M.Leprae*

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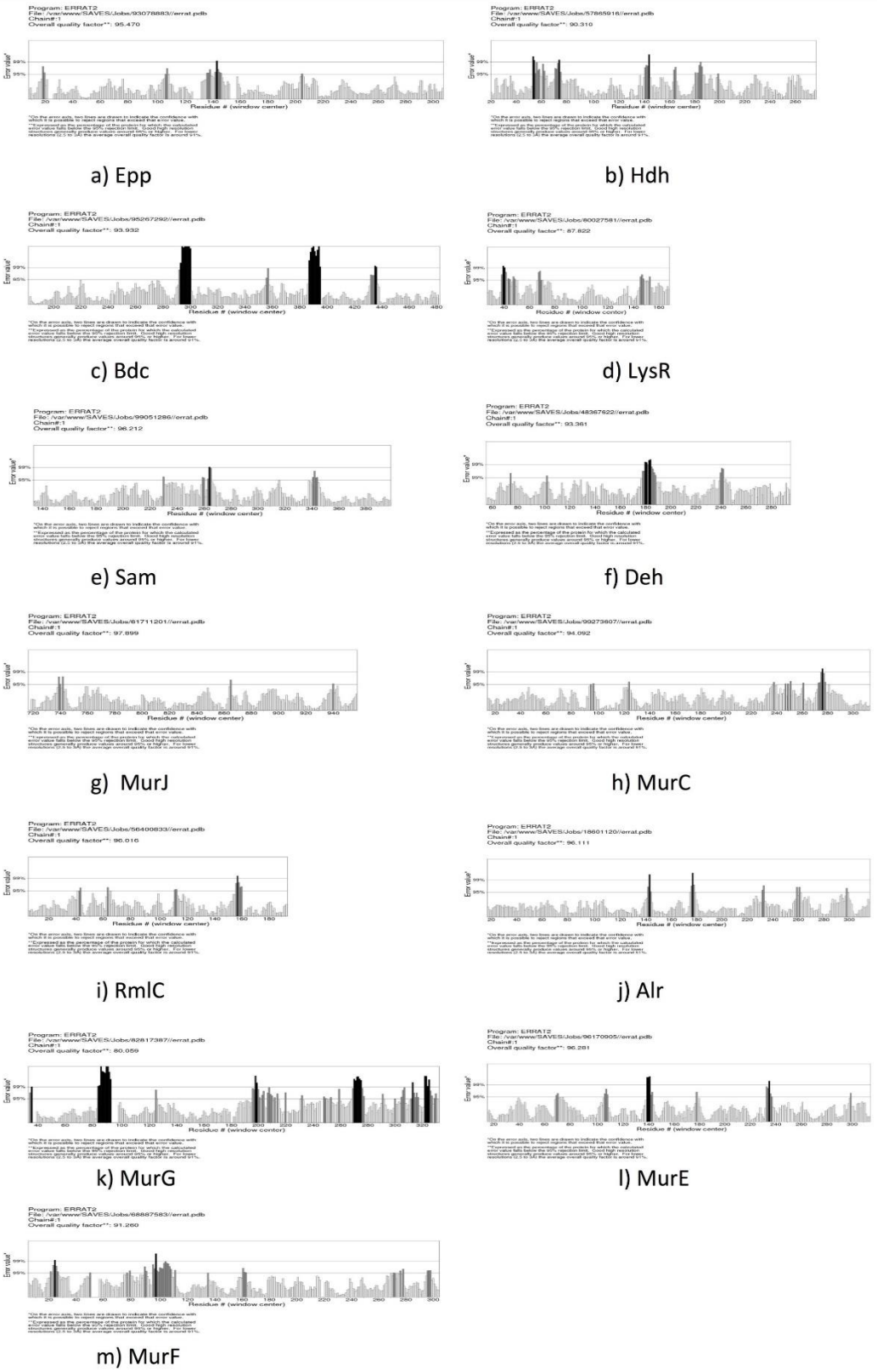

Fig 2- ERRAT plots for predicted 3D structures of proteins of *M.Leprae*

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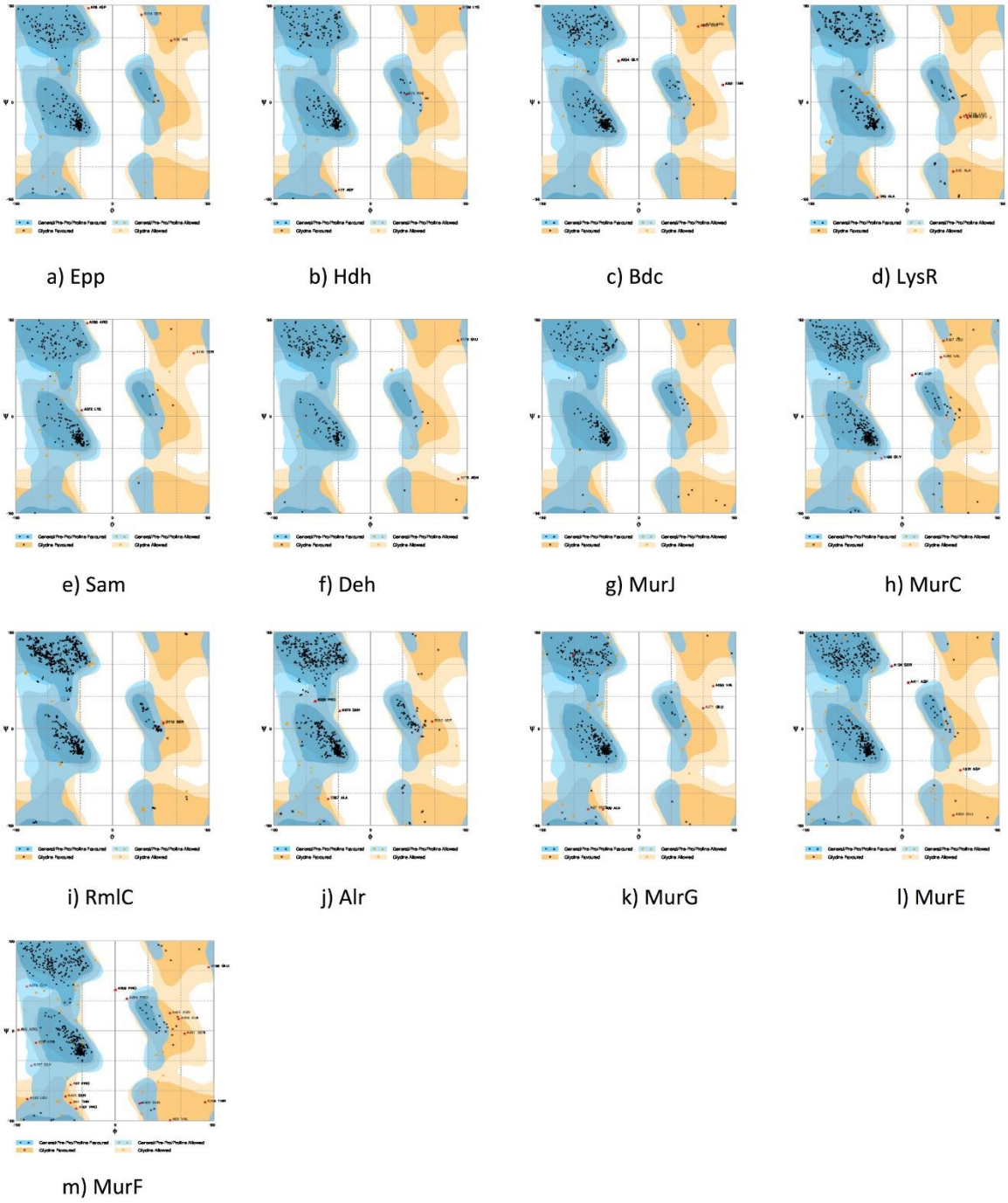

Fig 3- Ramachandran plots for predicted 3D structures showing the number of residues in favourable region.

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Molecular Docking

The target proteins were docked with the drugs and natural compounds to analyze their interactions and binding energy. Six chemical drugs and twenty natural compounds were taken from different medicinal plants which were known to treat leprosy were found through literature search. These were screened based on Lipinski Rule of Five to analyze the ADMET profile that gives details about the adverse properties of Absorption, Distribution, Metabolism, Excretion, and Toxicity of the compounds. The docking results of these proteins against different drugs were analysed. The top five compounds that interacted better with all the proteins under study were Hops extract, Daucosterol, Clarithromycin, Rifadin and Licoricidin. Out of all proteins, MurE and LysR family transcriptional regulator have a good fit with all the chemical compounds. Among all the compounds tested Hops extract from *Humulus lupulus* showed the strongest affinity against the protein MurE with a binding energy of -445.43. The mean binding energy for each chemical compound against all the proteins was highest for Hops extract from *Humulus lupulus*(-427.6007) and then to Daucosterol from *Justicia adhatoda* (-339.0207). The standard deviation of the individual compounds against all the proteins showed the lowest value for psorelen indicating that it interacts similarly with all the proteins under study whereas it is highest for glucuronic acid. The mean binding energies of an individual protein against various chemical compounds showed that LysR family transcriptional regulator (-282.80) and MurE (-271.73) have the highest values. So this analysis indicates that the proteins MurE and LysR family transcriptional regulator were found to be the most potent drug target for *M.Leprae*. Daucosterol from *Justicia adhatoda* and Hops extract from *Humulus lupulus* were found to be the most effective drug candidates against *M.Leprae* for the treatment of Leprosy.

Table 9- Top 5 compounds showing high fitness scores against target proteins of *M.Leprae*

Compounds→	Hops extract	Licoricidin	Daucosterol	Clarithromycin	Rifadin
Proteins					
Epp	-423.85	-313.33	-315.01	-319.98	-326.16
Hdh	-421.64	-304.01	-345.12	-314.49	-325.91
Bdc	-429.06	-298.97	-350.89	-298.11	-301.66
Lysr	-447.3	-339.78	-338.91	-350.39	-353.87
Sam	-442.75	-280.42	-320.97	-305.63	-303.25
Deh	-430.9	-323.15	-350.08	-308.3	-334.99
MurJ	-406.66	-288.35	-293.08	-284.43	-295.95
MurC	-442.57	-340.14	-366.75	-323.9	-313.17
RmlC	-417.26	-286.16	-322.89	-313.29	-310.08
Alr	-428.74	-286.68	-324.58	-367.68	-374.57
MurG	-417.17	-335.79	-380.77	-313.89	-322.84
MurE	-455.43	-366.39	-345.48	-331.82	-345
MurF	-395.48	-302.03	-352.74	-294.81	-304.55

CONCLUSION

The physiochemical characterisation of thirteen proteins under study gave a detailed insight of the properties like pI, EC, GRAVY, AI etc. Secondary structure analysis showed that the proteins have a dominating alpha helical structure which is responsible for their stability. Phosphorylation, glycosylation, myristoylation and amidation sites in protein structures indicate their high metabolic activity and also their thermal stability. The homology modelled 3D structures of all the proteins when evaluated using different servers were found to be of good quality. These structures were used in molecular docking with several market drugs and herbal compounds. The results showed Hops extract from *Humulus lupulus* has the best fit with almost all the proteins taken for study. The proteins LysR family transcriptional regulator and MurE were found to be the best targets against drugs for the treatment of leprosy. Thus the study was successful in predicting novel target proteins in *M.Leprae* MDR strains and also the best drugs to treat with. These proposed drugs were subjected to further experimental evaluation to effectively treat the disease and release for public use.

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