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RESEARCH ARTICLE

COMPUTER AIDED VIRTUAL SCREENING AND SELECTION OF NOVEL PHYTOLIGANDS AGAINST SHIGELLOSIS

Sinosh Skariyachan*[1] Navya Bharadwaj[1] and Nisha Prakash[1]

[1]R & D Centre, Department of Biotechnology, Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bangalore-560 078, India

* Department of Biotechnology, Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bangalore, India

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ABSTRACT

The outbreak of shigellosis, caused by a gram negative enteric pathogen-*Shigella sonnei*, has become a serious issue worldwide. IpgB1 is one of the major proteins involved in type III secretary pathway of the bacteria which mimics the role of Rho-G in membrane ruffling and the simulation of Rac-1. This condition leads to shigellosis and IpgB1 act as a key virulent factor. The infection usually treated by antibiotics; however, most of the strains acquired multiple drug resistance. Hence, there is a demand for alternative therapeutic agents. Computer aided screening is a modern approach to design next generation medicines. But the 3D structure of the protein is not available in native form which is essential for structure based drug designing. Hence, a 3D model of IpgB1 was generated by homology modeling. The model was refined and validated by various bioinformatics tools. The Ramachandran plot of the model accounts for 92.9% of the modeled residues are in allowed region. The model was used as a probable drug target and the inhibitory properties of selected ligands were studied by molecular docking. The binding efficiencies of known drugs were compared against selected herbal compounds. Herbal leads such as Andrographolide, Cryptolepine and Esculetin (docking binding energy -8.15 kcal/mol, -7.15kcal/mol and -6.79 kcal/mol respectively) were identified as the best inhibitors with minimum binding energies and good pharmacological properties than known chemotherapeutic agents. Present study finds significant application to design novel therapeutics against IpgB1mediated shigellosis

INTRODUCTION

Shigellosis is an acute bacterial infection of the lining of the intestines. The disease is mainly due to poor hygienic conditions, poor sanitation, and due to some types of sexual activities leads to atrocious, life threatening disease (Srinivasa *et al*., 2009). It is caused by any of the four subgroups of *Shigella* namely *S. sonnei, S. boydii, S. flexneri* and *S. dysenteriae*. Symptoms of shigellosis include acute abdominal pain or cramping, acute fever, blood, mucus, or pus in stool, crampy rectal pain (tenesmus), nausea, vomiting and watery diarrhea (Duric *et al*., 2009) . Shigellosis caused by *Shigella sonnei* emerged as a major public health burden, with more than 160 million cases and more than 1 million deaths every year, the majority of which occur in children from developing countries (Kotloff *et al*., 1999). Shigellosis is initiated once the *Shigella* invades into the colonic epithelium and lamina propria (Baron *et al*., 1996). Type III secretory systems present in the bacteria are mainly responsible for membrane ruffling. IpgB1 is one of the major proteins involved in this pathway which mimics the role of Rho-G in membrane ruffling. It belongs to a broad family of bacterial virulent factors that activate Rho-G signalling pathway (Huang & Chai, 2010). It also employs the RhoG-ELMO-Dock180 complex thus inducing the activity of Rac-1 thereby helping for the invasion of bacteria (Handa *et al*., 2007). Shigellosis is one of the major gastroenteritis throughout the world and severe infections may require antimicrobial treatment which can shorten the duration and severity of illness (Yadav *et al*., 2011). However, most of the strains developed multidrug resistance and selection of effective antimicrobial therapies became a tedious task (Niyogi, 2005). Computer aided method is a better and novel platform to study and rapid screening of potential inhibitors against drug targets. The 3D structure of the target, IpgB1 in the case of Shigellosis, is critical for structural drug designing and it is not available in native form. Hence, comparative modeling of IpgB1was carried out with a closely related template by Modeller 9V9. Modeller is a homology modeling software implements comparative structure modeling by satisfaction of spatial restraints and generates a good quality model (Marti-Renom *et al*., 2000). The study of receptor-ligand interaction is the fundamental rationale behind structure based drug design. Molecular docking is the best approach to study such kinds of interactions and it finds best binding affinities between receptor and ligand (Yuriev, *et al*., 2011). AutoDock is

***Corresponding author:** sinoshskariya@gmail.com*

advanced docking software relies on Lamarkian genetic algorithm which increases the execution speed and accuracy (Norgan *et al*., 2009). Docking predicts the binding energy of the protein with the ligand and calculates the score of each step based on which a suitable drug can be selected (Bikadi *et al*., 2004)

MATERIALS AND METHODS

Sequence retrieval of IpgB1 protein

The amino acid sequence of IpgB1 (Q3YTQ0) was retrieved from Uniprot (Apweiler *et al*., 2004) database and the sequence is used for present study.

Selection of best templates

A best homologous template is very essential for comparative modeling and the templates were selected by PSI BLAST (Altschul *et al*., 1997). The program compares protein sequences to sequence databases and calculates the statistical significance of matches. The tool produces a position specific scoring matrix constructed from a multiple alignment and constructs a profile. It is often used to detect relationships between proteins that are structural or functional homologues. The best templates were selected based on the percentage of identity, similarity, expected value and alignment scores.

Analysis of the conserved sequence and evolutionary relationship

Analysis of highly conserved amino acid residues is very essential to identify the functional domains and motifs. Multiple sequence analysis is the best approach to identify the conserved regions present in the targets and best templates. T-COFFEE (Notredame *et al*., 2000) is a multiple sequence alignment (MSA) program used to detect the conserved regions. The evolutionary relationship between the target and templates were further confirmed by phylogenetic analysis by NJ-Plot (Perrière *et al*., 1996). It is one of the simplest tools to visualize evolutionary distance between the sequences.

Proteomics characterization

The physiochemical analysis and secondary structure predictions are very essential for the characterization of the protein. The primary structure analysis of the toxin was carried out by ProtParam (Walker, 2005) which allowed the computation of various physical and chemical parameters such as molecular weight, isoelectric point, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity. The secondary structure of toxin was predicted by PSIPRED (McGuffin *et al*., 2000) which is an accurate secondary structure prediction method based on feed-forward neural networks. The pathogenecity island and functional domain of toxin was predicted by SMART (Schultz *et al*., 1998)

Homology modeling of the toxin

The crystal structure of drug target in the present study is not available in native form. Hence, the protein was modeled by homology modeling using Modeller 9v9 (Marti-Renom *et al*., 2000). It is based on satisfaction of spatial restraints derived from the alignment and probability density functions. The crystal structure of Transforming protein (Klink *et al*., 2010) RhoA (PDB ID: 3LW8, Chain E) was identified as the best template. The atom, alignment and script files were prepared in respective format. The initial model building and structural alignment was performed and the model was visualized by PyMOL (Seeliger *et al*., 2010)

Model refinement and validation

The homology model of the toxin is validated by various bioinformatics tools. Parameters such as covalent bond distances and angles; stereo-chemical validation and atom nomenclature were performed by PROCHECK (Laskowski *et al*., 1993) and the overall quality factor of nonbonded interactions between different atoms types were calculated by ERRAT (Colovos *et al*., 1993). The superimposition and structural alignment between the template and target was carried out by DaliLite (Holm *et al*., 2000) and the RMS values of the backbone alignment of alpha carbon atoms were also calculated. The model is further refined by Verify 3D (Eisenberg *et al*., 1997), What Check (Hooft *et al*., 1996) and PROVE (Pontius *et al*., 1996). The refined model was deposited to Protein Model Data Base (Castrignanò *et al*., 2006); the database collects 3D protein models obtained by structure prediction methods.

Screening of potential ligands and molecular docking

Since many strains of *Shigella sonnei* have developed resistance to conventionally used drugs, it is essential to screen other novel therapeutics. It has known that many herbal compounds have high inhibitory activity against several bacterial toxins. By extensive literature studies potential inhibitors were identified. The 3D structure of the all those molecules were retrieved from Pubchem (Wang *et al*., 2010), DrugBank (Wishart *et al*., 2008), KEGG (Kanehisa *et al*., 2000), Chemspider (Williams *et al*., 2010) databases and the druggish features were analyzed by Lipinski rule of five (Giménez *et al*., 2010). The rule stated that poor absorption or permeation of the drug is more likely when there more than 5 hydrogen bond donors and10 hydrogen bond acceptors, the molecular weight is over 500, the Clog P is over 5 and the sum of N's and O's is over 10. Five known drugs and herbal ligands were screened and docked with the modeled toxin by AutoDock 4.02 (Morris *et al*., 2010). The program uses a Monte Carlo simulated annealing for configurational exploration using grid based affinity potentials and provides bioactive conformation by energy minimization. The potential inhibitors were selected based on the binding energy, number of hydrogen bonds involved and cluster RMSD value.

RESULTS AND DISCUSSION

The protein sequence of IpgB1 of *Shigella sonnei* was retrieved from Uniprot (accession number: Q3YTQ0). The toxin has 208 amino acids and it is the major virulent factor for shigellosis. The FASTA format of the sequence was used as the target sequence for comparative modeling.

> sp|Q3YTQ0
MQILNKILPQVEFAIPRPSFNSLSYNKLVKKILSVFNLKQ
RFPQKNFGCPVNINKIRDVIDKIKDSNSGNMSQERTSYV
SSMINRSIDEMAIHNGVVLTSDNKKNIFAAIKFPDIKLDE
KSAQTSISHTALNEIASSGAKILKRYSSNMDLFNTQMKD
LTNLVSSSDKIFNESTKVLQIEISAEVLKAVYRQSNTN

Table 1: Best homologous template structures of ipgB1 toxin are screened by PSI-BLAST. Transforming Protein Rho A (3LW8_E) is identified as the best template for comparative modeling

PDB ID	Name of the protein	Name of the organism	Chain	Length of amino acids	Identity percentage	Similarity percentage	E-value
3GCG	MAP & CDC42 complex	*Escherichia coli*	B	172	28	49	9e-08
3LW8	Transforming protein RhoA	*Shigella flexneri*	E	192	27	50	2e-04
2K2O	Myoferlin (DysF domain)	*Homo sapiens*	A	123	24	43	0.14
1X9D	ClassI α-1,2-Mannosidase	*Homo sapiens*	A	538	32	47	3.1
3I0X	8-oxoguanine glycosylase/lyase	*Clostridium acetobutylicum*	A	291	22	44	6.8
3A7M	Flagellar type III chaperone	*Salmonella typhimurium*	A	122	30	53	7.5
2FPQ	Botulinum Neurotoxin Type D	*Clostridium botulinum*	A	444	46	65	9.9

Table 2: The pharmacological and druggish properties of selected lead molecules. The drugs were screened based on extensive literature survey and Lipinski rule of five

Compound ID (PubChem)	Name of the ligand	Origin	Molecular formula	Molecular Weight (g/mol)	Drug violation (Lipinki Rule)	XLogP	H-Bond Donor	H-Bond Acceptor
5318517	Andrographolide	Herbal	$C_{20}H_{30}O_5$	350.449	0	2.2	3	5
34458	Canadine	Herbal	$C_{20}H_{21}NO_4$	339.385	0	3.1	0	5
19009	Palmatine	Herbal	$C_{21}H_{22}NO_4^+$	352.403	0	3.7	0	4
82143	Cryptolepine	Herbal	$C_{16}H_{12}N_2$	232.279	0	3.3	0	1
5281416	Esculetin	Herbal	$C_9H_6O_4$	178.141	0	1.2	2	4
5273569	Fraxetin	Non Herbal	$C_{10}H_8O_5$	208.171	0	1.2	2	5
5273569	Lemofloxacin	Non Herbal	$C_{17}H_{21}F_2N_3O_3$	208.167	0	-0.9	2	8
5323714	Furazolidone	Non Herbal	$C_8H_7N_3O_5$	225.158	0	-0.1	0	6
5578	Trimethoprim	Non Herbal	$C_{14}H_{18}N_4O_3$	290.317	0	0. 9	2	3
115162	Melysin	Non Herbal	$C_{21}H_{34}ClN_3O_5S$	476.029	0		1	6

Table 3: Binding energy (kcal/mol) of various lead molecules with IpgB1 toxin after molecular docking. Herbal leads such as Andrographolide and Esculetin are interacted to the toxin by maximum number of hydrogen bonds and lowest binding energies implies these leads have better inhibitory activities than known drugs

Name of the ligand	Binding energy of the ligand (Kcal/mol)	RMSD value of the ligand	No. of hydrogen bonding interactions	Residues involved in H bonding
Andrographolide	-8.15	0.0	3	LYS 124, LYS 128, ARG 84
Esculetin	-6.79	0.0	3	SER157, ASN158, GLN10
Cryptolepine	-7.16	0.0	1	LYS 30
Canadine	-5.63	0.0	1	AGR81
Palmatine	-5.58	0.0	1	LYS124
Fraxetin	-4.59	0.0	1	GLU184
Lemofloxacin	-5.17	0.0	1	GLU184
Furazolidone	-5.25	0.0	1	LYS31
Trimethoprim	-4.10	0.0	1	GLU184
Melysin	-3.51	0.0	1	LYS124

The best homologous templates were identified by PSI BLAST search against PDB database. The best template proteins were screened based on the parentage of identity, alignment scores, E-value and the query coverage (Table 1). Among them, the template 3LW8 chain E (Transforming protein Rho-A of *Shigella flexneri*) was selected as the best homologous structure. It is an X-ray crystallographic structure of 192 amino acids and shared 27% identity with IpgB1 protein. It consists of 57% helical structures (7 helices of 111 residues) and 43% extended sheets (3 strands of 24 residues) .The resolution factor of the structure was found to be 1.85 A^o. A multiple sequence alignment was performed to identify the conserved regions and structural motifs. It is very significant as the structural motifs constitute the function of the toxin and the information is useful to study the receptor ligand interaction. The multiple alignments performed by T-COFFE shows the amino acids represented in the asterisk symbol have high conservation and identity. These regions constitute the virulent genes of the toxin (Fig.1A). The templates selected by BLAST were further confirmed by multiple alignment and phylogenetic analysis. It is evident that if the target sequence sharing homologous relationship with the template both of them would bifurcate from a common ancestor in a phylogram. Our studies indicated that transforming protein Rho-A of *Shigella flexneri* (PDB: 3LW8_E) showed maximum evolutionary relationship with IPGB1toxin. Hence, the structure can be used as the best homologous template for modeling The three dimensional structure of toxin was predicted from its basic amino acid sequence. Hence, the basic characteristics studies of amino acid residues are very

```
3LW8_E|          GAMDMLGTSFNNFGISLSHKRYFSGKVDEIIRCTMGKRI-----------
Q3YTQ0|1pgB1     --MQILNKILPQVEFAIPRPSFNSLSYNKLVKKILSVFNLKQRFPQKNFG
                   *::*.. : :. :::.:  : * . :::::  :.

3LW8_E|          VKISSTKINTSILSSVSEQIGENITDWKNDEKKVYVSRVVNQCIDKFCAE
Q3YTQ0|1pgB1     CPVNINKIRDVIDKIKDSNSGNQLFCWMSQERTSYVSSMINRSIDEMAIH
                   :. .**.  * .  ..: *:::  * .:*:. *** ::*:.**::. .

3LW8_E|          HSRKIGDNLRKQIFKQVEKDYRISLDINAAQSSINHLVS---GSSYFKKK
Q3YTQ0|1pgB1     NGVVLTSDNKKNIFAAIK-FPDIKLDEKSAQTSISHTALNEIASSGLRAK
                 :.  : .: :*:**  ::    *.** ::**:**.* .    .** :: *

3LW8_E|          MDELCEGMNRSVKNDITSNVANLI--SDQFFEKNVQYIDLKKLRGNMSDY
Q3YTQ0|1pgB1     ILKRY-SSNMDLFNTQMKDLTNLVSSSDKIFNESTKVLQIE-ISAEVLKA
                 : :   . * .: *   .:::**:  **::*::..: :::: : .:: .

3LW8_E|          ITNLESPF
Q3YTQ0|1pgB1     VYRQSNIN
                 : . ...
```

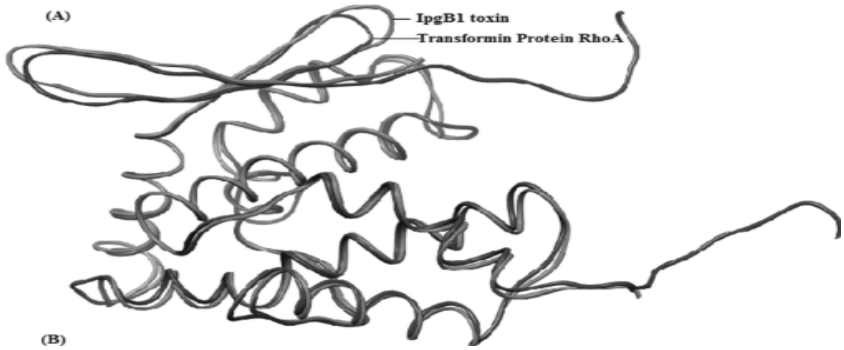

Fig. 1: Selection of best template. **(A)** Sequence alignment between 1pgB1 toxin and its best homologous template .The symbol '* 'indicate high conservation, ':' indicate high similarity and '.' Indicate less similarity. The alignment shows that the toxin has a high homologous relationship with its template structure. **(B)** The structural alignment of target and template was performed by superimposition techniques. The backbone of target is threaded against the E chain of Transforming protein Rho A. Exact superimposition of target and template indicating high homologous relationship. Hence, building of the three dimensional model of unknown protein is accurate and reliable.

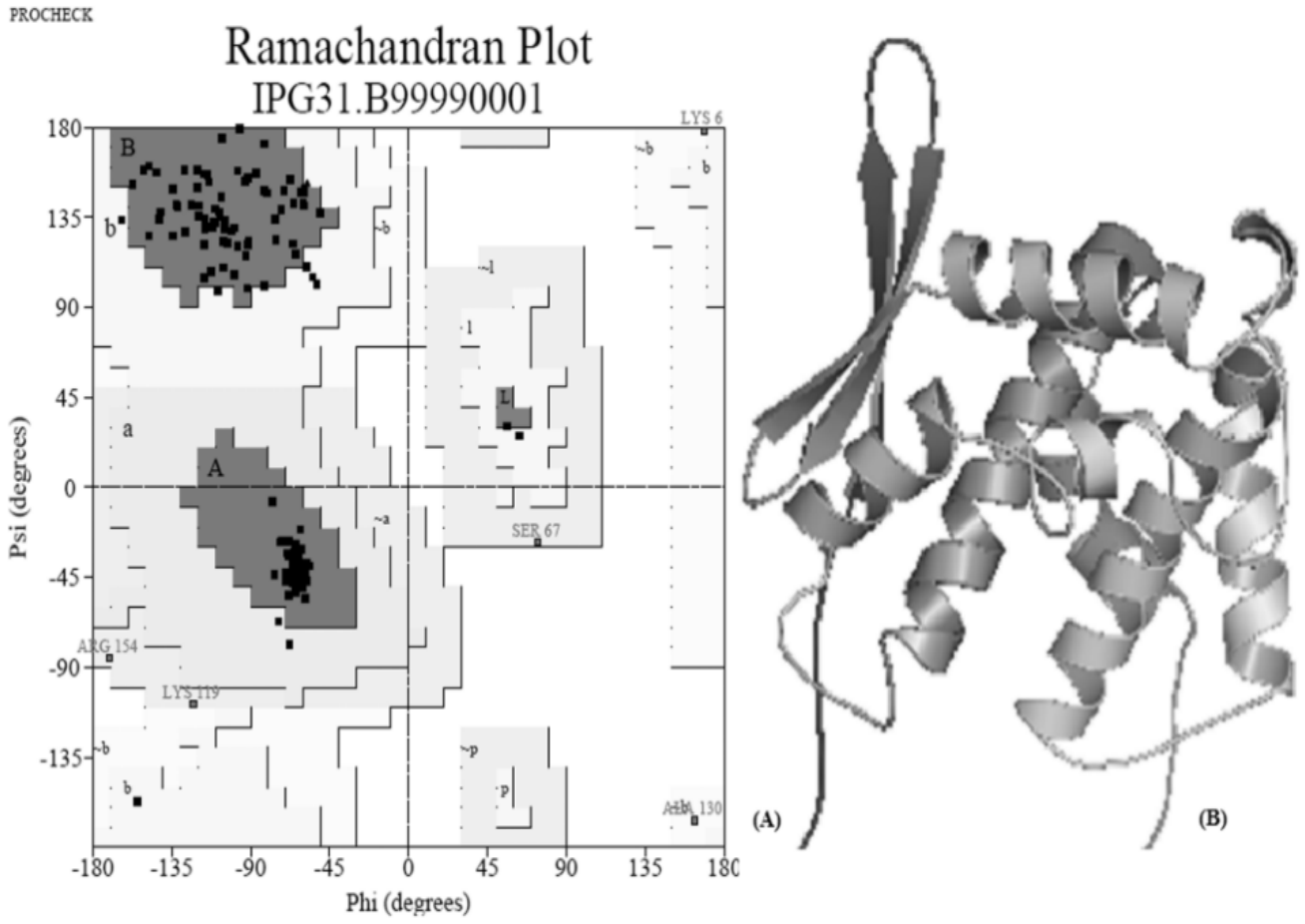

Fig. 2: Model refinement and steriochemical validation of modeled protein. **(A)** The Ramachandran plot of the model depict 92.9% of residues are located in the allowed region, 4.6% in the additionally allowed region, 2.6% in the generously allowed region implies good quality of the model. **(B)** The three dimensional structure of the modeled protein is visualized by PyMOL. The protein contains six α-helical domains and one β-domain, regions codes the pathogenic island of the toxin.

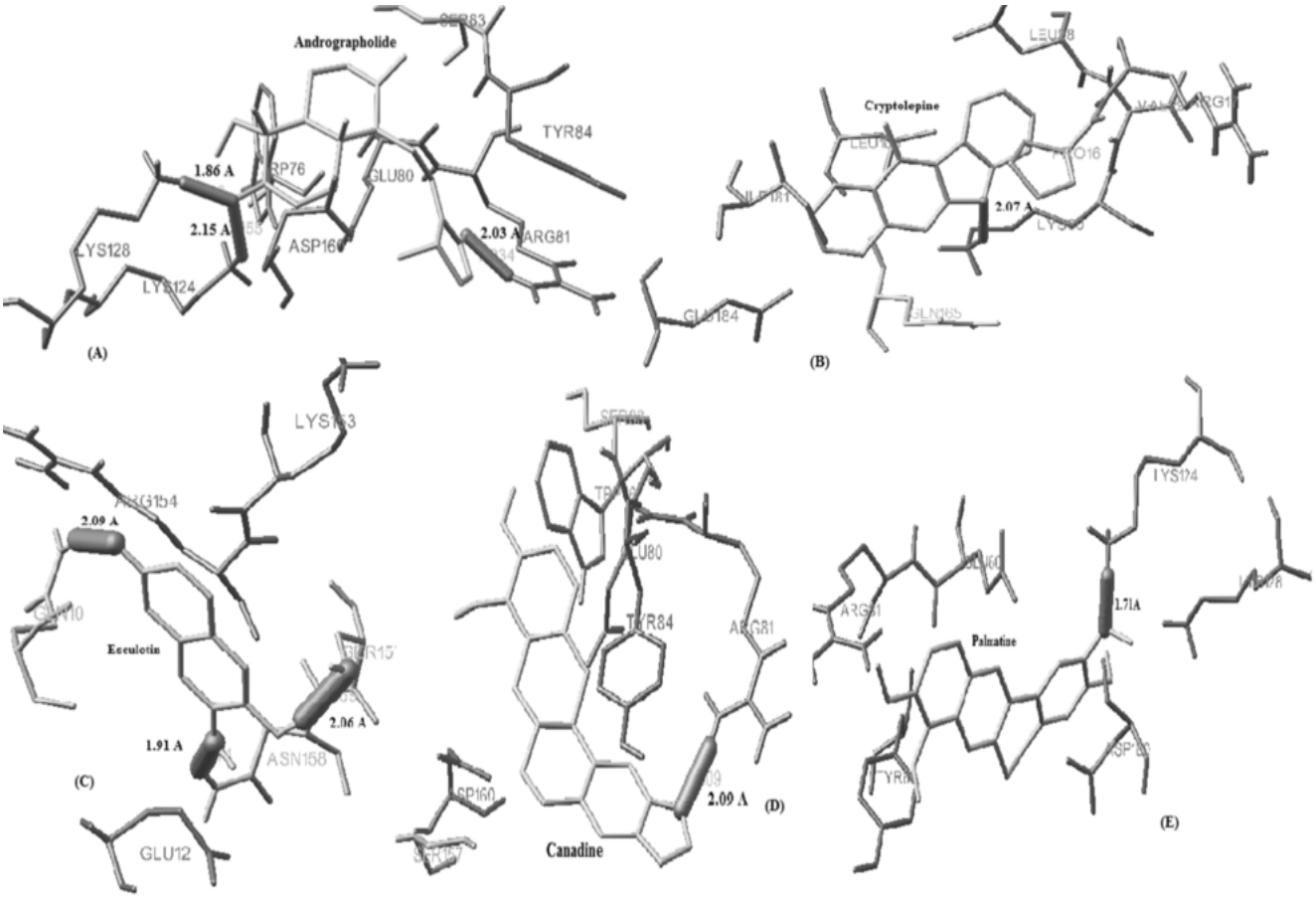

Fig. 3: Molecular interactions of various herbal inhibitors against ipgB1 toxin of *Shigella sonnei*. The amino residues present in the binding site of the toxin and the ligands are labeled and represented as stick figures. The hydrogen bonds are also represented in thick stick figures. The interacting residues and bond lengths are labeled in each interaction. **(A)** Interaction of herbal drug Andrographolide (binding energy -8.15 kcal/mol) is identified as the best ligand and the interaction is stabilized by three hydrogen bonds. **(B)** Herbal compound Crypolepine is interacted with the toxin by a hydrogen bond (binding energy -7.16 kcal/mol) and **(C)** Esculetin (binding -6.79 kcal/mol) interacted by three hydrogen bonds. The interaction of Canadine **(D)** and Palmatine **(E)** are stabilized by one hydrogen bond each (binding energies -6.79 kcal/mol and -6.79 kcal/mol respectively). The binding efficiency of known chemicals are also studied (not shown in the figure) and compared with herbal lead molecules. Present study revealed that herbal leads are better candidates for designing novel therapeutic agents against IpgB1 mediated *Shigella* infections.

significant. The primary structure of toxin is analyzed by Protparam. The tool allows the computation of various physical and chemical parameters of particular sequence. The molecular weight and isoelectric point were found to be 23674.2 Dalton and 9.60 respectively. The aliphatic index was found to be 92.31 and the grand average of hydropathicity was found to be -0.346. The toxin has 12 % serine and 10% Lysine and Aspargine. The total numbers of negatively charged residues (Asp + Glu) and positively charged residues (Arg + Lys) are 19 and 29 respectively.

The secondary structure of the protein was predicted by PSIpred revealed that 54.33% of alpha helix, 11.54% of extended strand, 1.92% beta turn, and 32.21% of random coil. The functional motif of IpgB1 was predicted by SMART. The virulent genes are located between 43 to 199 with a domain accession number PF03278. The domain is present in many gram negative pathogens and it plays a major role in gastroenteritis. Since the 3D structure of the toxin has not available in native form, a hypothetical model has generated by homology modeling. The modeled structure consists of seven α-helical domains and one β-sheet motif (Fig. 2B). These regions are crucial for the pathogenicity and functionality of the toxin. The structure was threaded over the backbone fragment of chain-E of transforming protein Rho-A to estimate RMSD (Fig.1B). The RMS value was found to be 1.0 A^o implies the quality of alignment between target and template is reliable. The Z-score of the alignment is found to be 26.8. The homology model was validated by SAVS server. The Ramachandran plot generated by PROCHECK showed that 92.9% of the residues in allowed region, 4.6% of the residue in the additionally allowed regions, and 2.6% in the generously allowed and none in disallowed region. The plot revealed the stupendous quality of our model (Fig. 2A) and the overall quality factor of non-bonded interactions between different atoms were identified as 58.03. The model was then submitted to Protein model database and it can be downloaded by the accession number PM0077551.

The study of receptor-ligand interactions are the fundamental principle of structure based drug discovery. We have used the modeled structure of toxin as the receptor. Five best chemical inhibitors herbal based lead molecules were identified by extensive literature survey and screened by Lipinski rule of five. The rule of five implies that molecules should contain less than 10 hydrogen bond acceptors and 5 hydrogen bond donors. The calculated logP value should be less than 5 and the molecular weight should be less than 500 g/mol. The 3D

structures of the drugs were selected from drug data bases mentioned earlier in the study. All selected molecules found to be qualified the rule of five (Table 2). Since computer aided docking is a reliable approach to study the receptor – ligand interaction, selected ligands were subjected to molecular docking by AutoDock 4.02. The scoring calculations were performed by Lamarkian genetic algorithm and molecular dynamics. The programme analyzed 27000 generations and evaluated 2500000 different conformations among 150 populations. The interaction between receptor and ligand was identified based on RMS clustering, number of hydrogen bond formation and binding energy. Minimum binding energy of the docked confirmation reveals stable interaction and good inhibitory properties.

Recent studies revealed that many strains of *Shigella sonnei* developed multiple resistances against many conventionally used antibiotics. Hence, the available medicines are no longer effective for the treatment for shigellosis. Many herbal drugs are known to have better inhibitory properties against many gram negative pathogenic bacteria. Hence, we have compared the efficiency of herbal drugs against currently available drugs. The selected ligands (both herbal derived compounds and currently used drugs) qualified the druggish properties were subjected to molecular docking. Our study revealed that the selected phytoligands have better inhibitory properties than known chemicals. Among herbal leads, Andrographolide, a labdane diterpenoid present in *Andrographis paniculata*, is interacted with the toxin by three hydrogen bonds with minimum binding energy of -8.15 kcal/mol. The interacting residues are LYS 124, LYS 128 and ARG 84 (Fig. 3A). Similarly, Cryptolepine (Figure 3B), a naturally occurring indoloquinoline alkaloid present in *Cryptolepsis triangularis* binding with LYS 30 of the toxin by a hydrogen bond (binding energy -7.16 kcal/mol). Esculetin, a phenolic compound found in *Cichorium intybus* and *Bougainvllra spectabillis* interacting with the toxin by three hydrogen bonds (Figure 3C). The binding energy of docked confirmation was estimated to be -6. 79 kcal/mol and interacting residues are SER157, ASN158 and GLN10. Similarly, Canadine, a natural isoquinoline alkaloid extracted from *Hydrastis canadensis* is interacted with AGR81 of toxin (binding energy -5.63 kcal/mol) by the formation of a hydrogen bond (Figure 3D). Palmatine, a protoberberine alkaloid present in *Phellodendron amurense*, *Rhizoma coptidis* and *Corydalis yanhusuo* is interacting with the toxin by a hydrogen bond (LYS124) and the energy of binding was estimated to be -5.58 kcal/mol (Figure 3E). The inhibitory properties of currently available drugs were found to less effective than herbal leads. The binding energies of the docked conformations of present generation drugs were found to be higher compared to herbal based lead compounds (Table 3). Our study concluded that herbal compounds such as Andrographolide, Esculetin and Cryptolepine have better inhibitory properties against IpgB1 toxin and these could be used as a novel therapy for shigellosis and similar types of toxin mediated gastroenteritis. Present data finds significant application in *in vitro* studies and the applied method pave a new therapeutic insight against shigellosis and similar types of gastroenteritis.

CONCLUSION

Shigellosis became one of the major health concerns throughout the world with high mortality rate. Our study concluded that computer aided method is an effective approach for screening of better remedies when current drugs seems to have failed. The approach is mainly target the key protein responsible for the disease by potential inhibitors. IpgB1 is one such protein causes membrane ruffles by stimulating the activities of Rac1 and Cdc42 and promotes the invasion of bacteria to the epithelial cells and results shigellosis. The 3D model of IpgB1, very essential for *in silico* studies, is not available in native form. Hence, the structure has modeled and validated by homology modeling. Several naturally available herbal compounds are identified and their binding properties were studied by molecular docking. The study concluded that herbal ligands such as Andrographolide, Cryptolepine, Esculetin, Canadine and Canadine have good inhibitory properties against the toxin and these could be used as the potential therapy against IpgB1 mediated shigellosis. The present findings have crucial applications in further laboratory studies.

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REFERENCES

[1] Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.

[2] Apweiler, R., Bairoch, A., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R, Magrane, M., Martin, M. J., Natale, D. A., O'Donovan, C., Redasch, N., Yeh, L. S. 2004. UniProt: the Universal Protein Knowledgebase. Nucleic Acids Res., 32: 115-119.

[3] Baron, S. Medical Microbiology, 4th ed.; Galveston (TX): University of Texas Medical Branch at Galveston: Texas, 1996.

[4] Bikadi, Z., Hazai, E. 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform., 1:15.

[5] Castrignanò, T., De Meo, P. D., Cozzetto, D., Talamo, I. G., Tramontano, A. 2006.The PMDB Protein Model Database. *Nucleic Acids Res*., 34: D306-D309.

[6] Colovos, C., Yeates, T. O. 1993. ERRAT: An Empirical Atom-Based Method for Validating Protein Structures, Protein Sci., 2: 1511-1519.

[7] Duric, P., Stefanovic, S., Petrovic, V., Cosic, G. 2009. Characteristics of shigellosis outbreaks in the AP of Vojvodina. Med Pregl., 62: 308-313.

[8] Eisenberg, D., Luthy, R., Bowie, J. U.1997. Verify3D: assessment of protein models with three-dimensional profiles, Methods Enzymol., 277: 396-404.

[9] Giménez, B. G., Santos, M. S., Ferrarini, M., Fernandes, J. P. 2010. Evaluation of blockbuster drugs under the rule-offive. Pharmazie., 65:148-152.

[10] Handa, Y., Suzuki, M., Ohya, K.., Iwai, H., Ishijima, N., Koleske, A. J., Fukui, Y., Sasakawa, C. 2007.*Shigella* IpgB1 promotes bacterial entry through the ELMO-Dock180 machinery. Nat Cell Biol., 9: 121-128.

[11] Holm, L., Park, J. 2000. DaliLite workbench for protein structure comparison. Bioinformatics., 16: 566-567.

[12] Hooft, R. W., Vriend, G., Sander, C., Abola, E. E. 1996. Errors in protein structures. Nature. 381: 272-272.

[13] Huang, Z., Chai, J. 2010. Mapping the selection mechanisms by bacterial GEFs. Virulence., 1: 93-96.

[14] Kanehisa, M., Goto, S. 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res., 28: 27-30.

[15] Klink, B. U., Barden, S., Heidler, T. V., Borchers, C., Ladwein, M., Stradal, T. E. B., Rottner, K., Heinz, D. W. 2010. Structure of *Shigella* IpgB2 in complex with human Rho A: implications for the mechanism of bacterial guanine nucleotide exchange factor mimicry. J. Biol. Chem., 285: 17197-17208

[16] Kotloff, K. L., Winickoff, J. P., Ivanoff, B., Clemens, J. D., Swerdlow, D. L., Sansonetti, P. J., Adak, G. K., Levine, M. M. 1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull. World Health Organ., 77:651–666

[17] Laskowski, R. A., Macarthur, M. W., Thornton, J. M. 1993. PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Cryst., 26: 283–291.

[18] Marti-Renom, M. A., Stuart, A., Fiser, A., Sánchez, R., Melo, F., Sali, A. 2000. Comparative protein structure modeling of genes and genomes. Annu. Rev. Biophys. Biomol. Struct. 29: 291-325.

[19] McGuffin, L. J., Bryson, K.., Jones, D. T. 2000. The PSIPRED protein structure prediction server. Bioinformatics, 16: 404-405.

[20] Morris, G. M., Goodsell, D. S., Huey, R., Olson, A. J. 1996. Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4. J. Comput. Aided Mol Des., 10: 293-304.

[21] Niyogi, S. K. 2005. Shigellosis. J. Microbiol., 43: 133-143.

[22] Norgan, A. P., Coffman, P. K., Kocher, J. P., Katzmann, D. J., Sosa, C. P. 2011. Multilevel Parallelization of AutoDock 4.2. J Cheminform., 3: 12.

[23] Notredame, C., Higgins, D. G., Heringa, J. 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol., 302: 205-217.

[24] Perrière, G., Gouy, M. 1996. WWW-Query: An on-line retrieval system for biological sequence banks. Biochimie., 78: 364-369.

[25] Pontius, J., Richelle, J., Wodak, S. J. Deviations from standard atomic volumes as a quality measure for protein crystal structures. J Mol Biol. 264: 121-136.

[26] Schultz, J., Milpetz, F., Bork, P., Ponting, C. P. 1998. SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci U S A., 95: 5857-5864.

[27] Seeliger, D., de Groot, B. L. 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J. Comput. Aided Mol. Des., 24: 417-22.

[28] Srinivasa, H., Baijayanti, M., Raksha, Y. 2009. Magnitude of drug resistant shigellosis: A report from Bangalore. Indian J. Med. Microbiol., 27: 358-360.

[29] Walker, J. M. 2005. The Proteomics Protocols Handbook, 1st ed.; Humana Press Inc., Totowa.

[30] Wang, Y., Bolton, E., Dracheva, S., Karapetyan, K.., Shoemaker, B. A., Suzek, T. O., Wang, J., Xiao, J., Zhang, J., Bryant, S. H. 2010.An overview of the PubChem BioAssay resource. Nucleic Acids Res., 38: D255-D266.

[31] Williams, A. J., Tkachenko, V., Golotvin, S., Kidd, R., McCann, G. 2010. ChemSpider - building a foundation for the semantic web by hosting a crowd sourced data basing platform for chemistry, J. Cheminform,. 2: O16.

[32] Wishart, D. S., Knox, C., Guo, A.C., Cheng, D., Shrivastava, S., Tzur, D., Gautam, B., Hassanali, M. 2008. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res.,* 36: D901-D906.

[33] Yadav, B.S., Tripathi, V., Kumar, A., Khan, M.F., Barate, A., Kumar, A., Sharma, B. 2011. Molecular modeling and docking characterization of Dectin-1 (PAMP) receptor of *Bubalus bubalis*. Exp Mol Pathol., 92: 7-12.

[34] Yuriev, E., Agostino, M., Ramsland, P. 2011.Challenges and advances in computational docking. J Mol Recognit. 24: 149-164.

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