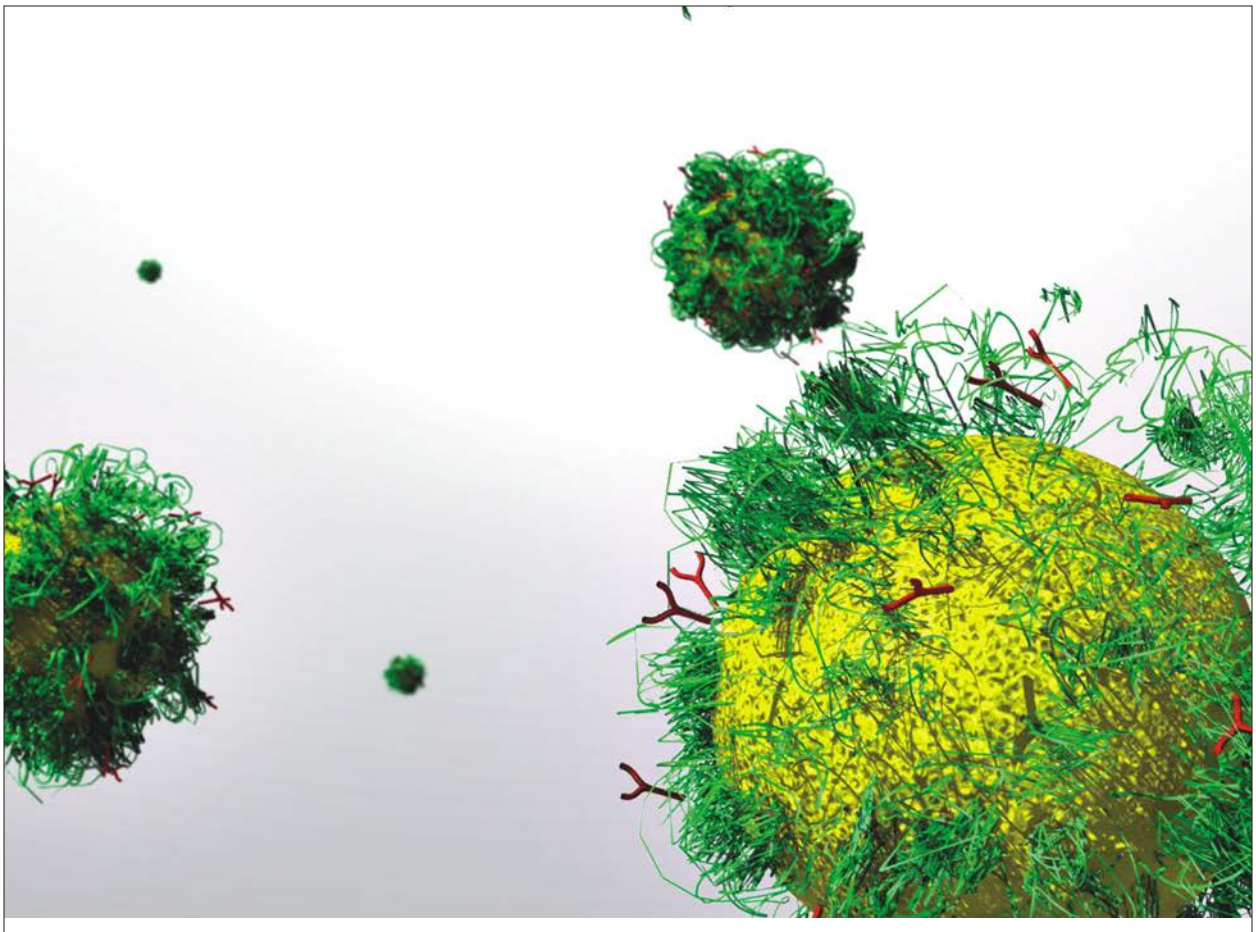

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In silico Analysis of Vi Polyssacharide Biosynthesis Protein of *Salmonella typhi*

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ABSTRACT

In developing countries, typhoid fever has a substantial negative socioeconomic impact. Highly invasive organism, *Salmonella typhi* is the causative agent for this enteric fever. The virulence of *Salmonella* is associated with the presence of a capsular polysaccharide, Vi antigen. The Vi polysaccharide biosynthesis protein, tviC, of *Salmonella typhi* whose three dimensional structure was not elucidated till date and its sequence was retrieved from KEGG database. Homology modeling was performed using Swiss model and the resultant structure was verified using WHATCHECK tool. Docking studies were done on the modeled structure with plant derived inhibitors such as allicin, apigenin, caffeic acid, curcumin, eugenol, piperin and luteolin. The hydrogen bond interactions between the protein and ligand were visualized by PYMOL software. Docking studies revealed the e-value for eugenol (-329) is better than the other selected ligands.

Key words: *Salmonella typhi*, docking, tviC, curcumin, e-value

INTRODUCTION

Salmonella typhi (*S. typhi*) is the etiological agent of typhoid fever, a serious invasive bacterial disease of humans with an annual global burden of approximately 16 million cases, leading to 600,000 fatalities. *Salmonella typhi* is a gram negative, obligate parasite that has no natural known reservoir outside the humans. It is a motile facultative anaerobe which causes typhoid fever in human. Many *S. enterica* serovars actively invade the mucosal surface of the intestine but are normally contained in healthy individuals by the local immune defence mechanisms (Parkhil *et al*., 2001).

Typhoid fever is a systemic infection caused by the bacterium *Salmonella enterica* subspecies *Enterica* serotype *typhi* which is acquired by ingestion of contaminated food and water (Hornick and Woodward, 1966; Arunachalam *et al*., 2012). Mortality rates associated with typhoid fever vary from region to region, with highest reported from Indonesia, Nigeria, India and Malaysia (Miller *et al*., 1994; Thong *et al*., 2000). Emergence of multidrug resistance strain of *S. typhi* enhances its importance as a major pubhc health problem. Abdul and colleagues confirmed presence of a 20 kb plasmid, is responsible for multidrug resistance (Khan *et al*., 2000). *S. typhi* seems to be more susceptible to genetic rearrangements (Liu and Sander, 1995) than other serotypes. These arrangements alter the stability, survival and virulence of the bacteria. Serum resistance is a major factor of gram negative bacteria. By using the isogenic Vi^+ and Vi^- strains it was confirmed that the Vi antigen is necessary for the serum resistance in *Salmonella typhi*.

(Hashimoto *et al.*, 1993). Pathogenic bacteria possesss genes responsible for its virulence (Okamoto *et al.*, 2009). The virulence of *Salmonella* is associated with the presence of Vi antigen (Looney and Steigbigel, 1986).

The Vi antigen discovered by Felix and Pitt (1936), is a capsular polysaccharide found mainly in *Salmonella typhi* and *Salmonella paratyphi* C. Purified Vi antigen from *Salmonella typhi* linear homopolymer of variety O-acetylated at the C_3 position (Daniel *et al.*, 1989). The Vi antigen is a capsular polysaccharide expressed by *Salmonella typhi*, the agent of human typhoid fever. Purified Vi polysaccharide is also licensed as a typhoid vaccine (Zhang *et al.*, 2006). The Expression of this antigen is controlled by the viaA and viaB chromosomal loci. The viaB locus is composed of 11 genes designated tvi A-tviE (typhi Vi), vex A-vexE (Vi antigen export) and ORF11 (Hashimoto *et al.*, 1991). Protein encoded by the viaB locus are not only involved in Vi polymer synthesis and translocation of the polysaccharide to the bacterial cell surface but also in regulation of Vi antigen expression in *Salmonella typhi* (Hashimoto *et al.*, 1993).

Importance of computational biology in designing ligands has become crucial in the current drug discovery procedure. Homology modelling has proven to be the method of choice to generate a reliable 3D model of a protein from its amino acid sequence. Docking is the computational simulation of a candidate ligand binding to a receptor. Docking is used to predict the binding orientation of small drug candidates to their protein targets to predict the binding affinity and activity of the small molecule. Docking plays an important role in rational design of drugs (Kitchen *et al.*, 2004). Drug discovery is the ultimate goal of drug designing which is concerned with the development of compounds with specific pharmacological activity (Pugazhenthi and Rajagopalan, 2007). According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care, majority of which use plants or their active principles (Gupta *et al.*, 2005). Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. Also the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). The use of traditional medicine remain widespread in developing countries while the use of complementary medicine is increasing rapidly in developed countries (Oladunmoye, 2006). *Salmonella* is an ideal organism for the development of newer drugs as a number of molecular mechanisms contributing to pathogens have been elucidated.

The present reserch was conducted to study the Vi polysaccharide biosynthesis protein-tviC of *Salmonella typhi* and to elucidate its primary and secondary structural properties, phylogenetic analysis, *in silico* comparative modeling and to perform docking studies with plant derived inhibitors such as allicin, apigenin, caffeic acid, curcumin, eugenol, piperin and luteolin.

MATERIALS AND METHODS

To analyze the selected target protein sequence (Accession No. stt:t4351 tviC) various tools and software's were used. The sequence was retrieved from KEGG (Kyoto Encyclopedia for Genes and Genomes) Database. Protparam was used for calculation of the physico-chemical properties of the protein. The secondary structure of the protein was analyzed using SOPMA. The sequence was compared for detecting homologous sequences found in databases using BLAST (Basic Local Alignment Search Tool). Since, no structural information was available at PDB (Protein Data Bank), the modeling of the protein was done to deduce the three dimensional structure of the protein. The homology modeling was performed with Swiss model and the structure was validated using WHATCHECK tool. The 3D co-ordinate file was visualized and analyzed using Swiss PDB viewer. The plant derived compounds for docking studies were retrieved from PubChem database.

The docking was performed using Hex software. Hydrogen bond between ligand and the protein were detected using PYMOL. Whatif server was used to analyze the active site of the target protein.

RESULTS AND DISCUSSION

The target protein sequence selected for the study was tviC (Accession No. stt: t4351 tviC) which is a Vi polysaccharide biosynthesis protein from the microorganism *Salmonella typhi* which is a bioweapon (Al-Agamy, 2011). The similarity search for the sequence was performed using BLAST tool. The results indicated that 1SB8_A is a homologous sequence to the target, tviC and its structure was available in PDB. The physico-chemical properties of the protein revealed the number of aminoacids to be 348, molecular weight: 39502.5 and the theoretical isoelectric point as 6.12. The total number of positively charged residues (Asp+Glu) was 42 and the total number of negatively charged residues (Arg+Lys) was 39. The aliphatic index and the grand average of hydropathicity was 88.85 and -0.330, respectively. The instability index was found to be 37.96. This classifies the protein as stable (Table 1). The secondary structure prediction was done using SOPMA. It was found that alpha helix occurs most frequently 47.13% (Table 2).

The target protein (tviC) structure was deduced by homology modeling. The template protein (1SB8_A) structural information was obtained from PDB. The energy minimization value after modeling was found to be -20066.217 (Fig. 1).

The modeled structure was validated using Whatcheck and the Ramachandran plot was plotted (Fig. 2).

Table 1: Physico-chemical properties of tviC (aminoacid composition)

	Residues	
Aminoacid	No.	%
Ala (A)	25	7.20
Arg (R)	20	5.70
Asn (N)	21	6.00
Asp (D)	24	6.90
Cys (C)	3	0.90
Gln (Q)	11	3.20
Glu (E)	18	5.20
Gly (G)	20	5.70
His (H)	6	1.70
Ile (I)	23	6.60
Leu (L)	35	10.10
Lys (K)	19	5.50
Met (M)	2	0.60
Phe (F)	16	4.60
Pro (P)	12	3.40
Ser (S)	28	8.00
Thr (T)	20	5.70
Trp (W)	5	1.40
Tyr (Y)	20	5.70
Val (V)	20	5.70
Pyl (O)	0	0.00
Asx (B)	0	0.00
Glx (Z)	0	0.00
Xaa (X)	0	0.00

Table 2: Secondary elements of tviC

	Residues	
Aminoacid	No.	%
Alpha helix	164	47.13
310 helix	0	0.00
Pi helix	0	0.00
Beta bridge	0	0.00
Extended strand	45	12.93
Beta turn	12	3.45
Bend region	0	0.00
Random coil	127	36.49
Ambigous states	0	0.00
Other states	0	0.00

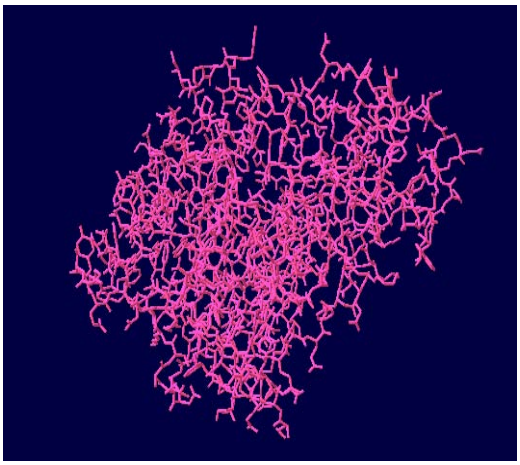


Fig. 1: Three dimensional structure of modeled tviC

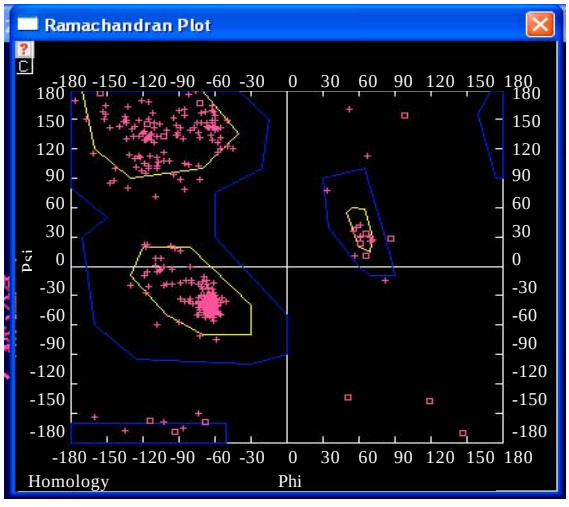
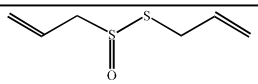
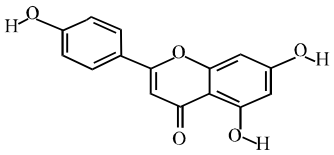
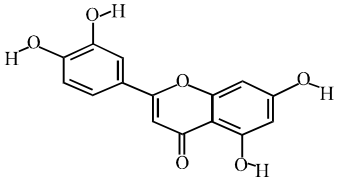
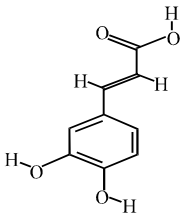
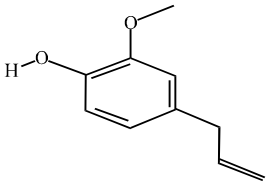
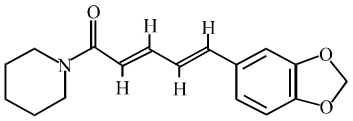
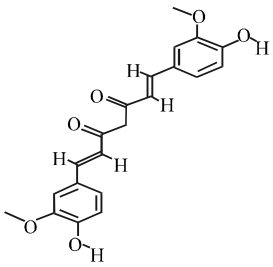


Fig. 2: Graphical representation of Ramachandran plot

Table 3: Plant derived inhibitors selected for docking

Inhibitors	Structure	Molecular weight (g moL^{-1})	Molecular formula
Allicin		162.273	$C_6H_{10}OS_2$
Apigenin		270.2369	$C_{15}H_{10}O_5$
Luteolin		286.2369	$C_{15}H_{10}O_6$
Caffeic Acid		180.15742	$C_9H_8O_4$
Eugenol		164.20108	$C_{10}H_{12}O_2$
Piperin		285.33766	$C_{17}H_{19}NO_3$
Curcumin		368.3799	$C_{21}H_{20}O_6$

The inhibitors chosen were plant secondary metabolites that were retrieved from PubChem database (Table 3). The choosen inhibitors are allicin, apigenin, luteolin, caffeic acid, eugenol, piperin and curcumin.

The selected inhibitors were docked with tviC and the docking results with its e-value were shown in Table 4. The hydrogen bond between inhibitors and the compound were calculated (Fig. 3).

The plant-derived medicines are relatively safer than synthetic drugs and offering profound therapeutic benefits by providing alternative and effective treatment for chronic disorders and various diseases (Barrett *et al*., 1999; Handa, 2004). The success of ethanobotanical approach to

Table 4: Docking results of tviC with the chosen inhibitor

Compound	E-value
Allicin	-151.2
Apigenin	-218.4
Caffeic acid	-169.4
Eugenol	-329.0
Piperin	-223.3
Curcumin	-257.3
Luteoline	-151.3

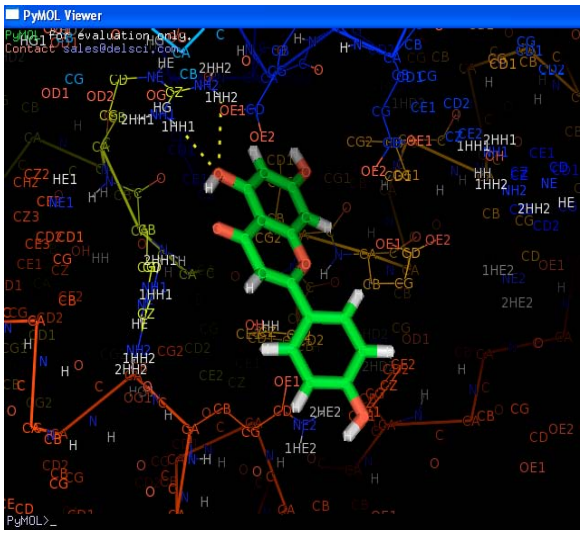


Fig. 3: Hydrogen bond between apigenin and tviC

drug discovery is highly promising (Abukakar *et al.*, 2008). The plant extracts and secondary metabolites possess antimicrobial, antifungal or antiviral activities (Mitscher, 1978). Allicin in its pure form, exhibit anti-bacterial activity against a wide range of gram negative and gram positive bacteria including *Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Streptococcus mutants* and *Bacillus cereus* (Miron *et al.*, 2000). The Energy value obtained after docking with allicin was -51.2. Apigenin shows *in vitro* antimicrobial properties by agar disc diffusion method against gram positive bacteria *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus* and gram negative bacteria *Escherichia coli, Pseudomonas aureginosa* and *Salmonella typhi* and antifungal activity against *Aspergillus niger* and *Candida albicans* (Devi *et al.*, 2010). The Energy value obtained after docking with apigenin was -218.4.

The *in vitro* studies provide evidence that luteolin and caffeic acid are potentially rich source of antimicrobial agent against microorganisms like *S. aureus*, *S. typhi* and *P. aeruginosa* (Padmini *et al.*, 2010). The energy value obtained after docking with luteolin and caffeic acid -151.3 and -169.4, respectively. Treatment with eugenol at their MIC (0.0125%) and MBC (0.025%) reduced the viability and resulted in complete inhibition of the organism, *Salmonella typhi* within 60 min exposure (Devi *et al.*, 2010). The Energy value obtained after docking with eugenol was -329. The antibacterial activity of piperin (black pepper) indicate excellent inhibition on the growth

of gram negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* at 250 ppm (Karsha and Lakshmi, 2010). The Energy value obtained after docking with piperin was -223.3. Curcumin biotransformed compound inhibited all of test bacterias and the most sensitive bacteria were *Salmonella typhi* with 9.0 mm inhibitory zone diameter (Rahman and Nur, 2009). The curcumin extract in combination with other plant extracts shows zone of inhibition ranging from 8-15 mm in case of *S. typhi* (Neogi *et al*., 2007). The energy value obtained after docking with curcumin was -257.3. The result of docking via Hex software reveal that eugenol has a better e-value than the other compounds.

CONCLUSION

Salmonella typhi virulence gene (tviC) was retrieved for target analysis. The primary structural informations of tviC are analyzed by using protparam tool which computed different parameters including molecular weight as 39502.5, the total No. of atoms as 13389, the isoelectric point is 6.12. The result indicates tviC protein was nearly neutral in nature. The secondary structural elements have been retrieved from SOPMA tool which shows nearly 47.13% was alpha helix. The tviC protein contains 164 alpha helices, 45 extended strands, 12 beta turns and 127 random coils. Through, BLAST search the template with 99% identity and 3e-125 e-value was found. The homology modeling of the selected target protein was done using swiss model. The energy minimization value after modeling was found to be -20066.217. The Ramachandran plot was also constructed. Modelled structure was validated using WHATHECK tool. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values (Nostro *et al*., 2000). The compounds such as Allicin, apigenin, piperin, curcumin, eugenol, luteolin and caffeic acid were retrieved from PubChem data base. Docking analysis with these compounds was done by using Hex 6.2. The protein-ligand interaction plays a significant role in structural based drug designing. The hydrogen bond interactions between the protein and ligand were visualized by PYMOL software. Docking studies reveals that the e-value for eugenol (-329) is better than the other selected ligands.

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