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# **Computational Characterization of Human Vascular Endothelial Growth Factor Proteins**

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## ABSTRACT

We present an in silico learning method to discriminate the pathologically important vascular endothelial growth factor (VEGF) protein through proteomic tools. Primary structure analysis showed most of the VEGF human proteins are rich in hydrophilic residues. The average molecular weight of VEGF human proteins calculated as 76244 Dalton. Grand Average hydropathy (GRAVY) index of all the VEGF human proteins are ranging from -0.2 to 0.1 except the protein 014495 which has comparatively high GRAVY value. Antigenic sites for all the proteins are recognized as C, Y, L, V, P, and K residues-EMBOSS antigenic program. The computed pI value indicates that most of the proteins are basic (pI>7) in nature. SOPM and SOPMA program shows that all the VEGF human proteins are different in secondary structural content. The presence of disulfide bridges are identified by CYS\_REC tool, also visualized through 3D structure. The SOSUI server classifies the proteins P15692, P49765 and 043915 as soluble proteins and other proteins as transmembrane proteins. The projected technique provides more accurate information about 3D structure, geometry, cystines involved in the disulfide bond. It would provide biological insights about protein hubs and their roles in interaction networks.

**KEY WORDS:** VEGF PROTEINS, PROTEOMIC TOOLS, HOMOLOGY MODELING, TRANSMEMBRANE PROTEINS, DISULFIDE BRIDGES.

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#### **INTRODUCTION**

Over the past few decade the occurrence of chronic diseases was increased due to change in life style, exposed to carcinogens that results in endothelial damage and failure to repair these injuries are the main cause of vascular injuries. Endothelial damage led to development of pulmonary disease, cancer and cardiovascular diseases. In all such chronic diseases, angiogenesis growth factor plays a predominant role. It is a physiologic process it involves formation of new blood vessels from pre-existing microvasculature (Goldmann, 1907, Okada, 2014; Yang, et al; 2017; Hulse, 2017).

The importance of this process widely known to be essential for growth of developing organs, wound healing, ovulation and pregnancy (Alitalo & Carmeliet, 2002). Ocular conditions related to angiogenesis also the leading cause of irreversible vision loss (Penna et al., 2008). Vascular endothelial growth factor (VEGF) - a dimeric glycoprotein and platelet derived growth factor (PDGF) have critical role in tumor-associated angiogenesis (Rivera & Bergers, 2015), cardiovascular diseases (Anthony Ware & Michael Simons, 1997) and principal causes of blindness (Penna et al., 2008). Where, the increase in vascular permeability to plasma proteins, induction of endothelial cell division and migration are widely reported in tumor angiogenesis (Dvorak, 2002; Hicklin & Ellis, 2005). About five glycoproteins are considered as the family of VEGF (VEGF-A, VEGF-B, VEGF-C, VEGF-D/F14F) and placental growth factor (PlGF) (Lee, Ellis & Daniel, 2008; Dvorak, 2002; Hicklin & Ellis, 2005).

VEGF-A isoform known to play a dominant role in solid tumors. The activation of VEGF ligands happens through binding to type III receptor tyrosine kinases, designated VEGFR1 (FLT1), VEGFR2 (KDR) and VEGFR3 (FLT4) (Waltenberger et al., 1994; Hiratsuka et al., 1998). The functional diversity of each tyrosine kinases depends on binding to specific receptors. Where, the expression of VEGFR1 is on vasculature and the exact role in vascular endothelium remain to be elucidated. Both VEGFR1 and VEGFR2 selectively expressed on vascular endothelial cells. In case of VEGFR3 has fundamental character in lymphatic system and embryogenesis (Laakkonen et al., 2007). In addition to these receptor tyrosine kinases (RTKs), VEGF interacts with a family of co-receptors, the neuropilins. The neuropilin receptor (NRP-1) expressed on vascular endothelium and neurons.

Table 1. Human VEGF protein sequences retrieved from Swiss-Prot Knowledgebase							
Accession Number	Entry name	Protein Names	Gene names	Length			
014495	LPP3_HUMAN	Vascular endothelial growth factor and type I collagen-inducible protein (VCIP)	PPAP2B (LPP3)	311			
014786	NRP1_HUMAN	Vascular endothelial cell growth factor 165 receptor	NRP1 (NRP) (VEGF165R)	923			
060462	NRP2_HUMAN	Vascular endothelial cell growth factor 165 receptor 2	NRP2 (VEGF165R2)	931			
P58294	PROK1_HUMAN	Endocrine-gland-derived vascular endothelial growth factor (EG-VEGF)	PROK1 (UNQ600/PRO1186)	105			
P15692	VEGFA_HUMAN	Vascular endothelial growth factor A (VEGF-A) (Vascular permeability factor) (VPF)	VEGFA (VEGF)	232			
P49765	VEGFB_HUMAN	Vascular endothelial growth factor B (VEGF-B) (VEGF-related factor) (VRF)	VEGFB (VRF)	207			
P49767	VEGFC_HUMAN	Vascular endothelial growth factor C (VEGF-C) (Vascular endothelial growth factor-related protein) (VRP)	VEGFC	419			
043915	VEGFD_HUMAN	Vascular endothelial growth factor D (VEGF-D)	FIGF (VEGFD)	354			
P17948	VGFR1_HUMAN	Vascular endothelial growth factor receptor 1 (VEGFR-1) (Vascular permeability factor receptor)	FLT1 (FLT) (FRT)	1338			
P35968	VGFR2_HUMAN	Vascular endothelial growth factor receptor 2 (VEGFR-2)	KDR (FLK1)	1356			
P35916	VGFR3_HUMAN	Vascular endothelial growth factor receptor 3 (VEGFR-3)	FLT4	1298			

VEGF-B (also called VEGF-related factor/VRF) is expressed more abundant in the heart and in the skeletal muscle cells (Olofsson et al., 1998). VEGF-C, it produced as a single propeptide, the N-terminal and C-terminal ends are proteolytically processed to generate a protein with high affinity for VEGFR-2 and VEGFR-3. VEGF-C induces mitogenesis and migration of EC (Kukk et al., 1996). In this regard, identifying specific regions is fundamental for scientific disciplines that require detailed characterization of proteins to explain essential biological systems. The biochemical and physicochemical characterization of VEGF proteins have not been done so far. By characterizing the VEGF proteins, we can impact our understanding of the relationship between protein flexibility and function. From this investigation, we report for the computational analysis and characterization of 11 Human VEGF proteins using proteomic tools and online prediction servers.

Proteomic tools and methods: Protein sequence retrieval and selection: Vascular Endothelial Growth Factor (VEGF) human proteins were retrieved from the UniProtKB/ Swiss-Prot release 57.0 (http://www.expasy.org/sprot) protein sequence database. The Swiss-Prot database was scanned for the keyword "vascular endothelial growth factor, Homo sapiens (Human) [9606]" through the search interface available in the Swiss-Prot database. The search yielded 65 proteins; all these protein sequences were downloaded in FASTA format (Lipman & Pearson, 1985). These 65 protein sequences were matched with each other using the online server "Blast 2 sequences" (http://www.ncbi.nlm.nih.gov/blast/bl2seg/wblast2.cgi) and finally 11 dissimilar protein sequences were selected for analysis. The details of vascular endothelial growth factor human proteins selected for analysis are tabulated in Table 1.

Table 2. Amino acid composition (in %) of VEGF proteins											
Amino				Acce	ssion Nu	mbers					
Acids	014495	014786	060462	P58294	P15692	P49765	P49767	043915	P17948	P35968	P35916
Ala	7	4	5	5	3	14	7	4	5	5	7
Cys	4	2	3	10	8	4	9	8	2	2	3
Asp	4	6	6	4	3	4	4	4	5	5	5
Glu	3	7	7	4	7	3	7	8	6	8	7
Phe	6	4	4	4	3	0	4	4	4	3	3
Gly	6	9	8	9	6	6	5	3	5	6	6
His	3	3	2	4	5	3	2	4	2	2	3
Ile	8	7	6	4	3	2	2	4	6	6	4
Lys	5	6	4	4	9	3	6	7	8	6	4
Leu	10	7	8	10	7	8	7	7	9	9	11
Met	3	2	2	4	3	2	2	2	2	2	2
Asn	5	5	4	3	3	0	5	3	4	4	3
Pro	5	6	6	6	6	13	6	7	5	5	5
Gln	3	3	4	1	6	6	5	4	3	4	4
Arg	6	4	5	10	8	6	6	7	5	5	6
Ser	8	8	9	6	6	8	8	10	10	8	8
Thr	5	6	6	6	3	6	6	6	7	7	5
Val	5	6	5	6	5	8	4	5	6	8	7
Trp	0	2	2	1	2	1	1	1	1	1	2
Tyr	5	4	4	1	3	1	3	1	4	4	3

**Proteomic tools and servers:** The amino acid composition of human vascular endothelial growth factor proteins were computed using the tool BioEdit 5.0.9 (Hall, 1999). Percentages of hydrophobic and hydrophilic residues were computed using the primary structural data. The physico-chemical parameters such as theoretical isoelectric point (pI), molecular weight, extinction coefficient (Gill & Von Hippel, 1989), half-life (Bachmair et al., 1986; Gonda et al., 1989; Tobias et al., 1991; Ciechanover & Schwartz, 1989; Varshavsky, 1997), instability index (Guruprasad et al., 1990), aliphatic index (Ikai, 1980), antigenic site and grand average of hydropathy (GRAVY) (Kyte & Doolittle, 1982) values were computed using the Expasy's ProtParam prediction server (Gasteiger et al., 2005).

The correlation between the number of acidic and basic residues is calculated on this server. The SOPM and SOPMA tools were used for the secondary structure prediction (Geourjon, Deleage, 1994 & Geourjon, Deleage, 1995). Secondary Structural Content Prediction (http://coot.embl.de/SSCP/) server is used for the computation of percentages of  $\alpha$ -helical,  $\beta$ -strand (Eisenhaber et al., 1996). The SOSUI server (Takatsugu Hirokawa et al., 1998) allowed the identification of transmembrane regions in VEGF human proteins. The tool BioEdit was used to compute the Kyte and Dolittle mean hydrophobicity profile of the transmembrane regions (Hall, 1999). Multiple sequence alignment of transmembrane regions computed using the MSA tool

to generate the sequence logo of transmembrane regions (Lipman et al., 1989; Schneider & Stephens 1990). The ScanProsite tool was used to identify the profiles with a high probability of occurrence in the PROSITE database (Edouard de Castro et al., 2006; Falquet et al., 2002).

**Prediction of disulfide bridges-SS bound cysteines**: A disulfide bridge in VEGF proteins is predicted by two different methods. The first method defines the presence of disulfide bonds (SS) and total number of cysteines using the protein sequences (FASTA format) submitted in the CYS\_REC tool. In a second method SS bonds are identified through visualization of three-dimensional (3D) structure of proteins; the 3D structure of five proteins was predicted by homology modeling using the Esypred server and visualized in the RasMol tool.

Table 3. Hydrophobic and hydrophilic residues content						
Accession Number	Percentage of hydrophobic residues	Percentage of hydrophilic residues	Net hydrophilic residues content			
014495	54.7	45.3	Low			
014786	47.5	52.5	High			
060462	46.2	53.8	High			
P58294	53.3	46.7	Low			
P15692	43.5	56.5	High			
P49765	46.4	53.6	High			
P49767	45.3	54.7	High			
043915	40.4	59.6	High			
P17948	45.2	54.8	High			
P35968	46.8	53.2	High			
P35916	48.5	51.5	High			

**Structure analysis and validation:** For comparative modeling, the five protein sequences are selected based on disulfide bridges predicted in CYS\_REC tool. The similar 3D structures were predicted in Protein Data Bank (www.rscb.org) through BLASTP analysis with the expectation value of 0.01 for the 060462, P15692, P49765 & P58294 proteins. For, the other protein (014495) a similar 3D structure was predicted with expectation value of 10. The modeled 3D structures were validated using servers Rampage (Ramachandran plot), ProQ (Protein Quality server), and ProSA (Protein Structure Analysis) (Lovell et al., 2002; Cristobal et al., 2001; Wiederstein & Sippl, 2007).

### **RESULTS AND DISCUSSION**

Primary structure analysis suggest that most of the studied human VEGF proteins are hydrophilic in nature (Table 2 and Table 3) except 014495 (VEGF type I collagen-inducible) and P58294 (Endocrine-glandderived VEGF) protein. The amino acids asparagines, lysine, aspartic acid, glutamine, histidine, arginine and glutamic acid are responsible for hydrophilic property. The average molecular weight of VEGF human proteins calculated is 76244 Dalton. Hydrophilic molecules are polar charged residues and capable of hydrogen bonding, dissolve more readily in water than oil. As reported earlier, the beneficial effect of hydrophilic drugs (Statin & Provastatin) in decreasing hemodynamically compromising rejection, attenuation of rise in lipid profiles; also in favor of a reduction in allograft coronary artery disease (Mandeep R. Mehra et al., 2004) [34]. Followed by, the antigenic sites (Asite) for all the VEGF proteins are identified through EMBOSS antigenic program (table 2). It attempts to understand the role of protein structure and thermodynamics of protein interactions during pathological conditions through binding at specific epitopes.

Table 4. Physiochemical parameters computed using BioEdit, EMBOSS, and Expasy's ProtParam

1001											
Accession No.	Length	M.Wt.	pI	-R	+R	EC	HL (hours)	II	AI	GRAVY	A Site
014495	311	35116	9.31	21	35	29965	30	39.49	94.41	0.104	268C
014786	923	103134	5.58	117	94	156995	30	35.56	72.54	-0.441	880Y
060462	931	104858	5.04	126	87	175110	30	47.57	72.59	-0.47	889Y
P58294	105	11714	9.01	8	15	7615	30	40.64	77.05	-0.038	14V
P15692	232	27042	9.21	24	40	39055	30	52.3	57.54	-0.783	15L
P49765	207	21601	8.46	16	19	14480	30	59.27	75.51	-0.232	55P
P49767	419	46883	7.77	46	49	47755	30	58.1	57.09	-0.496	159V
043915	354	40444	8.16	43	48	36825	30	56.81	61.67	-0.556	274K
P17948	1338	150768	8.66	151	169	164500	30	46.14	82.19	-0.35	12C
P35968	1356	151526	5.6	172	145	178020	30	45.34	87.21	-0.273	415L
P35916	1298	145598	5.89	163	139	214235	30	49.19	85.79	-0.282	159L

M.Wt. - Molecular weight; pI - Isoelectric point; R - residues; EC - Extinction coefficient at 280nm; II - Instability Index; AI - Aliphatic Index; GRAVY - Grand Average Hydropathy; A Site - Antigenic Site. Table 5. Computed theoretical isoelectric point (pI) and number of acidic and basic amino acids

Accession Number	pI	No. of Basic Amino acids	No. of Acidic Amino acids	Property
014495	9.31	35	21	Basic
014786	5.58	94	117	Acidic
060462	5.04	87	126	Acidic
P58294	9.01	15	8	Basic
P15692	9.21	40	24	Basic
P49765	8.46	19	16	Basic
P49767	7.77	49	46	Basic
043915	8.16	48	43	Basic
P17948	8.66	169	151	Basic
P35968	5.6	145	172	Acidic
P35916	5.89	139	163	Acidic

Expasy's ProtParam computes the extinction coefficient (EC) for a range of (276, 278, 280 and 282nm) wavelength. The EC value at 280nm is favoured because proteins absorb strongly while, the other substances commonly in protein solutions do not. Extinction Coefficient (EC) of VEGF human proteins at 280nm is ranging from 7615 to 214235 M-1 cm-1 with respect to the concentration of Cys, Trp and Tyr (Table 4). Expasy's ProtParam classifies most of the VEGF human proteins as unstable on the basis of Instability index (II>40) and the two proteins (014495, 014786) as stable (II<40) proteins in the room temperature. The aliphatic index (AI) that is defined as the relative volume of a protein occupied by aliphatic side chains (Ala, Val, Ile and Leu) is regarded as a positive factor for increase of thermal stability of globular proteins is low (57-94) for all of the VEGF human proteins and it infers that the VEGF proteins may become unstable at high temperature.

Table 6. Percentage of residues forming alpha, beta, and coil structures

Accession Number	Alpha Helix	Extended Strand	Beta Turn	Random Coil	Class
014495	46.6	12.5	3.2	37.6	Mixed
014786	16.1	24.9	7.4	51.6	Beta
060462	14.5	24.0	7.3	54.2	Mixed
P58294	9.5	17.1	5.7	67.6	Beta
P15692	29.3	15.1	3.9	51.7	Alpha
P49765	28.0	10.1	5.8	56.0	Alpha
P49767	33.9	7.2	4.3	54.7	Alpha
043915	30.5	9.0	3.7	56.8	Mixed
P17948	21.5	23.1	4.9	50.5	Mixed
P35968	22.6	23.8	6.1	47.6	Mixed
P35916	22.4	23.0	5.9	48.6	Mixed

Table 7. Transmembrane regions identified using SOSUI server					
Accession Number	Transmembrane region	Туре	Length		
014495	VLLICLDLFC LFMAGLPFLIIET	Primary	23		
	NDAVLCAVGIV IAILAIITGEFY	Primary	23		
	IQNPYVAALYKQ VGCFLFGCAIS	Secondary	23		
014786	ERGLPLLCAVLA LVLAPAGAFRN	Primary	23		
	ILITIIAMSALGVL LGAVCGVVL	Primary	23		
060462	ITIIAMSSLGV LLGATCAGLLLY	Primary	23		
	MDMFPLTW VFLALYFSRHQ	Secondary	19		
P58294	GATRVSIMLL LVTVSDCAVITGA	Primary	23		
	RDVQCGAGTC CAISLWLRGLRMC	Secondary	23		
P15692	Soluble				
P49765	Soluble				
P49767	MHLLGFFSVA CSLLAAALLPGP	Primary	23		
043915	Soluble				
P17948	SYWDTGVLL CALLSCLLITGSSS	Primary	23		
	ELITLTCTC VAATLFWLLLTLFI	Primary	23		
P35968	IIILVGTAVI AMFFWLLLVIILR	Primary	23		
P35916	IVILVGTGVIA VFFWVLLLLIFC	Primary	23		
	GAALCLRLWLCL GLLDGLVSGYS	Secondary	23		

Grand Average hydropathy (GRAVY) index of all the VEGF human proteins are ranging from -0.2 to 0.1 and this indicates that all these proteins may interact equally and easily with water except the protein 014495 which has comparatively high GRAVY value. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of the protein is zero. At pI proteins are stable and compact. The computed pI value indicates that most of the proteins are basic (pI>7) in nature (Table 5). The number of basic and acidic amino acids in each VEGF human proteins correlates well with the pI computed. The computed isoelectric point (pI) will be useful for developing buffer systems for purification by Isoelectric focusing method. The computed protein concentration and extinction coefficients help in the quantitative study of protein-protein and protein-ligand interactions in solution.

**3.3. Secondary structure analysis:** The secondary structure was predicted for all the proteins using SOPM and SOPMA tools; it shows that all the VEGF human proteins have different secondary structural content. The computed percentage of residues forming  $\alpha$ -helices,  $\beta$ -strands and coils are shown in Table 6.

Table 8. Organization of profiles identified in the VEGF protein sequences						
Accession	Identified profiles	Domains				
014495	No hit					
014786		CUB domain Coagulation factors 5/8 type C domain				
060462		MAM domain CUB domain Coagulation factors 5/8 type C domain MAM domain				
P58294	No hit					
P15692	PDGF_2	Platelet-derived growth factor				
P49765	POGF_2	Platelet-derived growth factor				
P49767	PDOF_2	Platelet-derived growth factor				
043915	POF_2	Platelet-derived growth factor				
P17948		Ig-like domain Protein kinase domain				
P35968		Ig-like domain Protein kinase domain				
P35916		Ig-like domain Protein kinase domain				

The SOSUI server classifies the proteins P15692, P49765 and 043915 as soluble proteins and other proteins as transmembrane proteins. The various primary and secondary transmembrane regions identified by SOSUI server were shown in Table 7. The identified transmembrane regions were found to have more hydrophobic residues and it is well documented by the Kyte and Doolittle mean hydrophobicity profile (Fig.1) in which all the peaks are above the zero line. The sequence logo of transmembrane regions (generated from the multiple sequence alignment of transmembrane regions) is shown in Figure 2. The height of each letter in the sequence logo is proportional to the frequency of the amino acid at that position. The presence of more leucine amino acid in the transmembrane region is identified from sequence logo. Generally, the amino acid

Table 9. Presence of cysteine residues and disulphide bond patterns predicted by CYS\_REC tool and visualized by RasMol in 3D protein structures.

Accession	מחק	No. of	Disulfide	RasMol
Number	r DD templata	INU. UI	bridges	Kd5iVI01
Number	tempiate	Cystemes	CVS PEC	
014495	2AKC	11	Cys68-Cys162,	Cys38-
				Cys43,
			Cys171-Cys181	Cys132-
				Cys181
				Cys268-
				Cys269
014786	Too large	22	Too large	
060462	2QQJ	24	Cys28-Cys277,	Cys277-
				Cys427,
			Cys55-Cys927,	Cys434-
				Cys592
			Cys149-Cys646,	
			Cys230-Cys883,	
			Cys626-Cys892,	
			Cys655-Cys902,	
P58294	1IMT	11	Cys26-Cys38,	Cys26-
				Cys38,
			Cys32-Cys96,	Cys32-
				Cys50,
			Cys37-Cys78,	Cys37-
				Cys78,
			Cys50-Cys86,	Cys60-
				Cys86,
			Cys60-Cys80	Cys96-
				Cys80
P15692	3V2A	18	Cys52-Cys94,	Cys52-
				Cys94
			Cys77-Cys172,	Cys83-
				Cys128
			Cys83-Cys128,	Cys87-
			0	Cys130
			Cys86-Cys171,	
			Cys87-Cys130,	
			Cys184-Cys202,	
			Cys187-Cys204,	
			Cys206- $Cys225$ ,	
DADZCE		0	Cys213-Cys227	Care 47
F49/65	ZVVVE	ď	Cys72-Cys81,	Cys47-
			Curo70 Curo100	Cys89
			Cys78-Cys122,	Cys82-
			Currol 2 Com 10.4	Cys124
			Cyso2-Cys124	Cys78-
DADACA	Toolore	20	Toologra	Cys122
P49767	Too large	38	Too large	
U43915	Too large	30	Too large	
F1/948	Too large	<u>زر</u> در	Too large	
F 35968	Too large	<u>ک</u> ک ۲	Too large	
169561	100 large	35	100 large	

ProSA.							
Accession Number	Rampage Residues in Favored	ProQ LGscore Max Sub		ProSA Z Score	Protein quality		
	region (%)						
014495	88	0.96	0.059	-1.35	Fairly good model		
060462	95.9	4.740	0.292	-6.91	Extremely good model		
P58294	96.1	0.968	0.119	-5.08	Fairly good model		
P15692	97.8	1.210	0.133	-4.44			
P49765	96.8	2.083	0.239	-3	Very good model		







Figure 3: Homology modeling-3D structure of VEGF proteins (Ribbon) and Cysteines (SS) bonds (ball and stick) viewed by RasMol tool. A) 060462; B) P58294; C) P15692; D) P49765



Figure 2: Sequence logo representation of the (generated using the multiple sequence alignment) transmembrane regions.



Figure 4: Validation of 3D structure of VEGF proteins-Ramachandran plot. A) 060462; B) P58294; C) P15692; D) P49765.



Leucine has the capacity to stimulate protein synthesis in muscles (Etzel, 2004).

The Scanprosite server identified different profiles in VEGF proteins except the protein 014495 (Vascular

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endothelial growth factor and type I collagen-inducible protein [VCIP]), and P58294 (Endocrine-gland-derived VEGF protein). The organization of all the identified profiles is given in table 8.

Figure 5: Validation of 3D structure of VEGF proteins-ProQ. A) 060462; B) P58294; C) P15692; D) P49765.



The location of disulfide bridges in all the proteins are screened using CYS\_REC tool. VEGF proteins showed cysteine residues (~1-35) and the positions of most probable SS bond patterns are predicted. Disulfide bridges identified only in few proteins, the selection of pairs are skipped due to too large in SS-bound cysteines as shown in table 9. In another method, the cysteines and the SS bond positions are identified in 3D structure of proteins. Where, few proteins did not have probable SS bond patterns as visualized in 3D structure. There are few unpaired cysteines identified (Fig.3A,B,C & D).. The 3D structures of proteins are validated through Ramachandran plot, ProQ and ProSA, the score were within the acceptable limits (Table 10; Fig. 4 & 5) (Lovell et al., 2002; Cristobal et al., 2001; Wiederstein & Sippl, 2007). The profile of 3D VEGF protein structures match its own sequences with Z scores, it expresses backbone identical to that of the template. This means not only the positions of alpha carbons, but also phi and psi angles with secondary structure are identical to the template.

Prediction of disulfide bonds in these proteins able to show the stability and structure of proteins. It's a covalent bond between sulfur atoms that binds two peptide chains or different parts of individual peptide chain oxidized to create a stable R-S-S-R bond and is a structural determinant in many of the protein molecules. These are essential to antibodies in creating the cell-surface receptors for target cells, as well as being part of the surface receptors of cells.

# CONCLUSION

Sequence-based approaches have proven to be very useful for functional prediction, entire map of protein complexes, architecture of network and their cell signaling factors for known active sites or binding regions. This information would be integrated in various experimental conditions so that overall signaling networks are characterized which gives unique characteristics on targeted protein-drug interactions. Hence, the data and concepts discussed here offer a sense of direction for harnessing the proteomic tools towards protein characterization. Such knowledge may then be channeled to the development of improved targets for biomedicine in the near future. The present work paves the way towards meaningful new areas in which technologies may be further exploited, especially using proteomic tools in order to advance innovate therapies and diagnostics.

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