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In Silico Docking Analysis of Selected Anti-Oxidant Compounds Against VEGF Protein

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Abstract
Vascular Endothelial Growth Factor (VEGF) is a key regulator of vasculogenesis and angiogenesis. Although, expression of VEGF is necessary for body functioning but its overexpression supports oncogenesis. Therefore, it has become a major target for the development of cancer therapeutics. Various anti-VEGF compounds have been explored for anti-angiogenic properties and are presently under clinical trials. The present study is focused on *in silico* analysis of various known antioxidants against VEGF-A, using *i*GEMDOCK software. Hesperidin has shown minimum binding energy -121.04 kcal mol^{-1} amongst all antioxidants, followed by quercetin (-98.79 kcal mol^{-1}), gallic acid (-77.49 kcal mol^{-1}) and ascorbic acid (-68.42 kcal mol^{-1}). Hesperidin, a known anti-angiogenic compound, appeared to be the best inhibitor of VEGF-A, and may be further explored for development of anticancer drug.

Keywords- VEGF-A, *i*GEMDOCK, Antioxidants, *In Silico* Analysis, Hesperidin, Ascorbic Acid, Quercetin, Gallic Acid, Anti-Angiogenic.

1. Introduction

Angiogenesis is the formation of new capillaries from the existing vessels (Ferrara, 2004). It plays an important role in embryonic development, normal growth, restoration and healing of tissues. Angiogenesis supports survival of cancer cells through vascularization and supply of oxygen, hormones, nutrients and various growth factors (Hoeben et al., 2004). VEGF (Vascular Endothelial Growth Factor), FGF (Fibroblast Growth Factor) and PDGF (Platelet Derived Growth Factor) are some important growth factors which are the regulators of angiogenesis. VEGF, due to its central role in angiogenesis, has become an important target for the development of anti-cancer drug (Frumovitz and Sood, 2007; Meadows and Hurtwitz, 2012).

VEGF family has seven proteins, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PGF (Placental Growth Factor) and VEGF-F. Bevacizumab (from Genentech) is the first FDA (Food and Drug Administration, USA) and EMEA (European Medicines Agencies) approved anti-VEGF drug for the treatment of colorectal, breast, lung and renal cell cancers etc. Nine more chemical drugs are in the list of anti-VEGF drugs (Meadows and Hurtwitz, 2012). But still there is requirement of natural VEGF inhibitors due to side effects of synthetic drugs in

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cancer therapy (Hoeben et al., 2004). Hesperidin is known to inhibit vascularization by blocking the AKT/mTOR signaling pathways (Kim, 2015). Still the fundamental mechanism for the anti-angiogenic activity of hesperidin is not fully understood. VEGF-A is most studied VEGF factor which plays an important role in various diseases and is a key regulator of angiogenesis (Holmes and Zachary, 2005).

In the present study, *in sillico* docking analysis of VEGF-A was done with various known antioxidants like ascorbic acid, hesperidin, gallic acid and quercetin.

2. Materials and Method

2.1 Protein Structure

Sequence of VEGF-A protein (ID-P15692.2) was retrieved from NCBI database in FASTA format. Homology modeling was done using SWISS-MODEL, which is the most widely used server for the modeling of protein structure (Schwede et al., 2003). Validation of protein structure was done with the help of Ramachandran plot obtained through RAMPAGE server.

2.2 Ligand Structure

Ascorbic acid, hesperidin, gallic acid and quercetin were used as ligand in this study. The structure of antioxidants was obtained from ZINC database (Irwin and Shoichet, 2005).

2.3 Molecular Docking

Docking analysis was done with the help of *i*GEMDOCK version 2.1. Doxorubicin was used as control (Yang and Chen, 2004). All the ligands were docked against the receptor, VEGF-A.

3. Result and Discussion

3.1 Sequence Retrieval and Primary Sequence

Query sequence of VEGF accession ID (P15692.2) of 117 amino acids was retrieved from NCBI database. The amino acid sequence of VEGF-A is given in Figure 1.

>gi|17380528|sp|P15692.2|VEGFA_HUMAN RecName: Full=Vascular endothelial growth factor A; Short=VEGF-A; AltName: Full=Vascular permeability factor; Short=VPF; Flags: Precursor.
MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRSYCH
PIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQ
GQHIGEMSFLQHNKCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVYVG
ARCCLMPWSLPGPHPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERT
CRCDKPRR

Figure 1. Amino acid sequence of VEGF-A

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3.2 Homology Modeling of the Protein

Homology modeling of the protein was done with the help of SWISS MODEL software as shown in Figure 2.

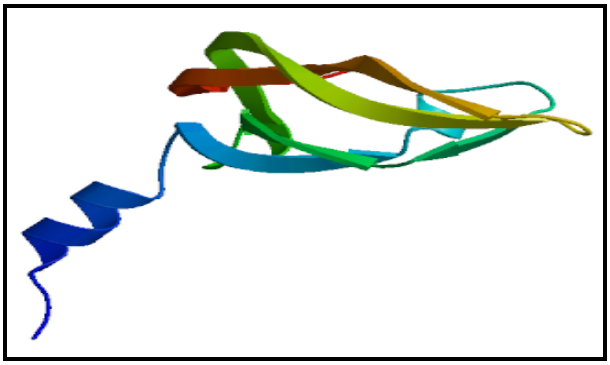

Figure 2. Homology model of protein VEGF

3.3 Protein Structure Analysis

RAMPAGE server was used for validation of protein models. RAMPAGE server analyzed protein structure's stereochemical stability with the help of Ramachandran plot. Ramachandran plot (Figure 3) illustrates that if more than 95% of amino acids residues fall in favored region then only protein will be stable and can further be used. In the present study the number of residues in the most favored region were 88 (94.6 %), numbers of residues in allowed region were recorded as 5 (5.4 %) and the numbers of residues in the outlier region were 0 (0 %).

Qualitative analysis of VEGF residue was analyzed through Verify3D software. Verify3D software gives a 3D-ID score on the basis of local environment of each residue. It provides all information on the basis of the area of the residue that is buried, the fraction of the side-chain area that is covered by polar atoms (oxygen and nitrogen), and the local secondary structure (Wallner and Elofsson, 2006). If a residue showed 3D-ID score above than zero than the model is in accepted phase. As shown in Figure 4, 3D-ID score of VEGF residue was found ≥ 0.2, so the model was in accepted phase.

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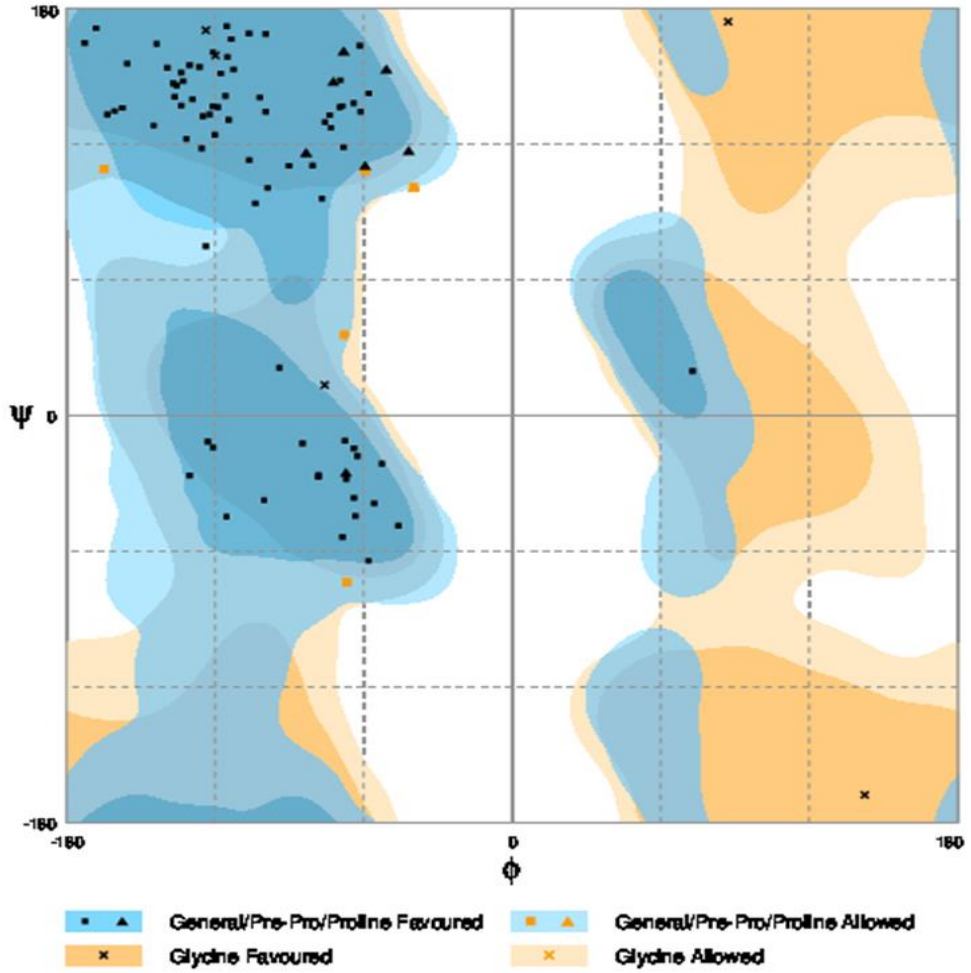

Figure 3. Assessment of protein stability of VEGF-A by Rampage

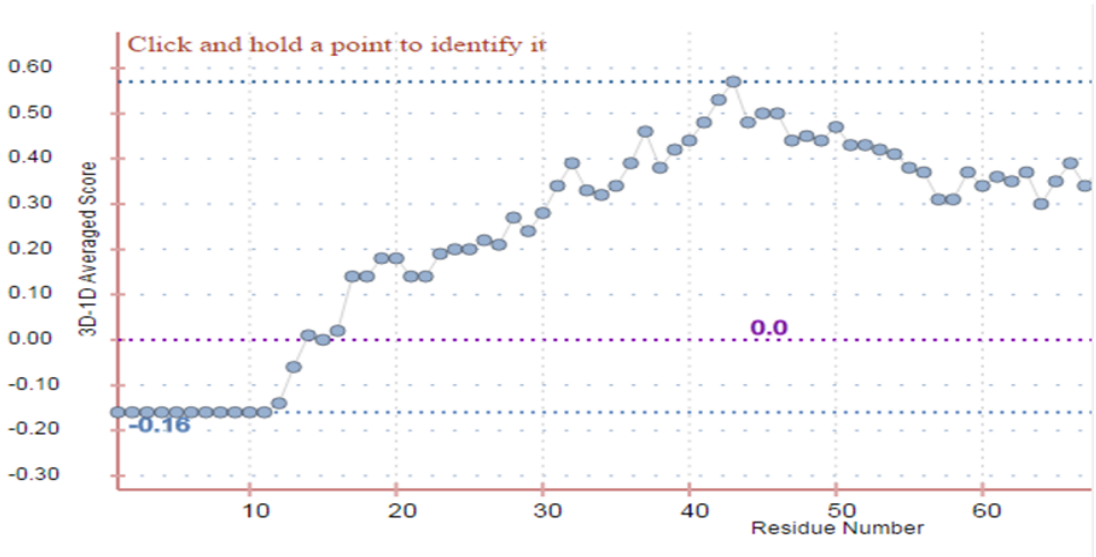

Figure 4. 3D Plots obtained from Verify 3D software for model based on the template of VEGF receptor used which recorded 75.79% of the residues had an average 3D-1D score ≥ 0.2

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3.4 Molecular Docking Analysis

Molecular docking was done with the help of iGEMDOCK software. Docking results of all lead compounds (antioxidants used) were compared with the control drug, doxorubicin (Table 1).

Table 1. Result of docking analysis

Compound Name	Energy (kcal mol^{-1})	VDW	HBond	Elec
Ascorbic acid	-68.42	-38.16	-30.37	0
Quercetin	-98.79	-72.46	-26.33	0
Gallic acid	-77.49	-52.44	-25.05	0
Hesperidin	-121.04	-87.89	-32.15	0
Doxorubicin	-112.75	-87.19	-25.56	0

As shown in Table 1, hesperidin has shown minimum binding energy (-121.04 kcal mol^{-1}) amongst all compounds used in docking with VEGF. Doxorubicin showed lesser binding energy (-112.75 kcal mol^{-1}) than hesperidin. The binding energies of quercetin, gallic acid and ascorbic acid were found to be -98.79 kcal mol^{-1}, -77.49 kcal mol^{-1} and -68.42 kcal mol^{-1}, respectively).

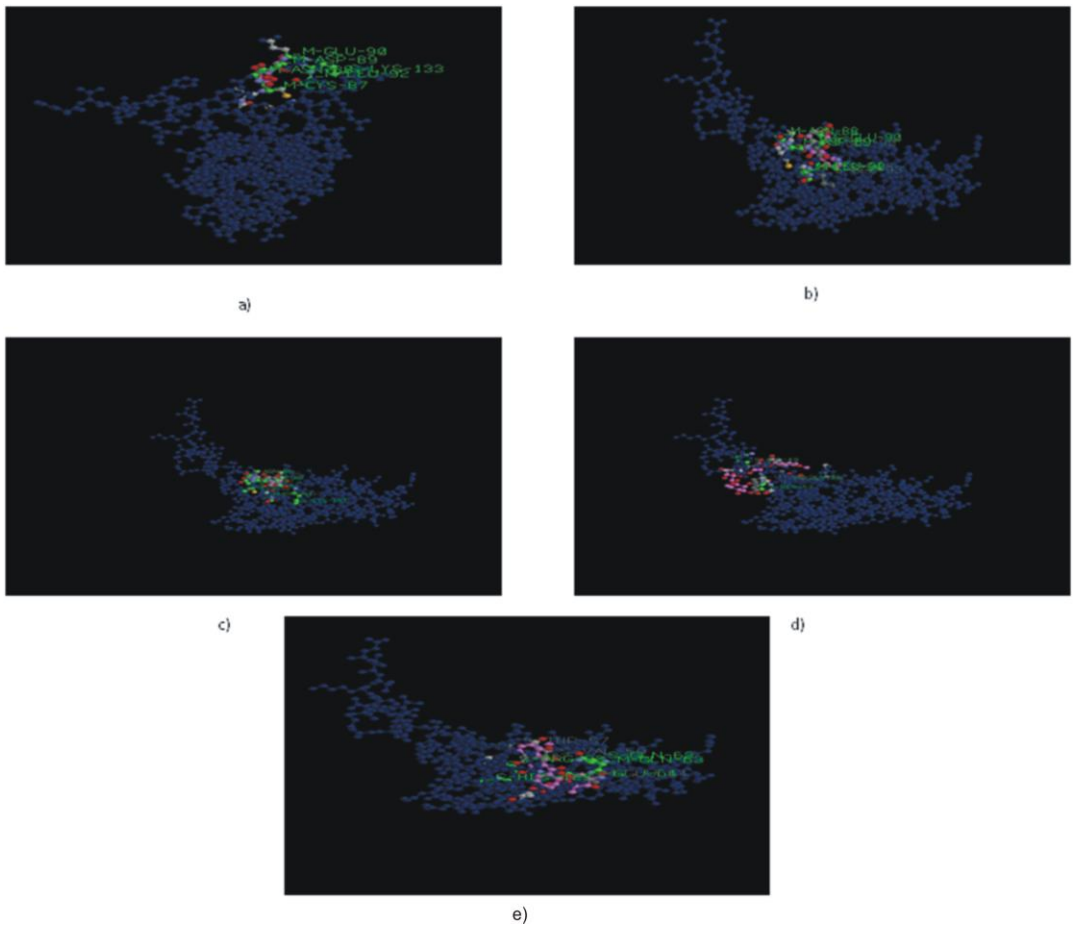

Figure 5. Docking images of lead compounds with VEGF. (a) Dock pose of ascorbic acid with VEGF. (b) Dock pose of quercetin with VEGF. (c) Dock pose of gallic acid with VEGF. (d) Dock pose of hesperidin with VEGF. (e) Dock pose of doxorubicin with VEGF

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Interacting sites of VEGF with different antioxidant compounds are shown in Figure 5 and Table 2.

Table 2. Common interacting site of VEGF with antioxidant compounds

Sr. No.	Docking compound	Interacting site in VEGF
1	Ascorbic acid	LYS 110, GLN 113, GLN 115,HIS 116
2	Quercetin	CYS 87, ASP 89, GLU 90, CYS 94, LYS 133
3	Hesperidin	CYS 87, ASP 89, GLU 90, LEU 92, CYS 94
4	Gallic acid	TYR 47, SER 50, TYR 51, CYS 86, ASN 88
5	Doxorubicin	SER 50, CYS 86, CYS 87, GLU 90

4. Conclusion

Hesperidin has shown minimum binding energy amongst all the compounds used. On the basis of *in sillico* analysis done through *i*GEMDOCK software. Hesperidin turned out to be the best anti-VEGF-A inhibitor amongst the others used in this study.

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