



GP and NPC1 Targeted Herbal Compound – An in Silico Evidence for Ebola Drugs

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Abstract

In silico based drug design is one of the potential techniques to discover new drug leads against essential drug targets. Ebola viruses (EBOV) of Filo viruses, consists of five species, viz. Zaire, Sudan, Ivory Coast, Bundibugyo, and Ravn, spreading hemorrhagic fatal fever worldwide. The reservoirs are yet to be confirmed, but the fruit bats have been considered to be the possible hosts for the serious transmissions. EBOV pathogenesis depends on viral recognition, attachment and transmission of virion to host cell and lysis. Recent research reveals that apart its own proteins (to be named, NP, VP35, VP40, glycoprotein (GP), sGP, VP30, VP24, and RNA-dependent RNA polymerase (L)), the EBOV use Niemann-Pick C1 for the transmission. In this study, a preliminary assessment of the natural compounds (from Ayurvedic plants) based on bioavailability related criteria, were docked with potential drug targets of Ebola virus. Out of the seventeen leads, six (neoandrographalide, fumaric acid, vasicoline, andrographalide and andrograpanine) showed prominent binding to active sites of GPs and NPC1s proteins. These drugs bind to the residues responsible for native conformation of the viral proteins (GPs and NPC1s). Since, the natural compounds show minimal side-effects compared to the synthetic ones, the use of these compounds or formulations possessing them through a proper delivery platform or as leads for future drugs will upgrade the mode of Ebola treatment.

Keywords: Ebola; Ayurveda; Glycoprotein; NPC1; Andrographalide; Docking

Introduction

The most complex Ebola outbreak of 2014 caused widespread pathogenesis and spread of the virus. As of September 2015, the Ebola virus outbreak in Western Africa has claimed more than 11,000 lives and more than 28,000 infections accounting to 40% case fatality rate (Figure 1) [1]. Ebola belongs to the virus family of Filoviridae and five species have been identified: Zaire, Bundibugyo, Sudan, Reston and TaI forest. Zaire species has been responsible for the 2014 West African outbreak [2].

Ebola virus (EBOV) is a membrane enveloped filamentous virus that contain a negative sense single stranded RNA. Genomic and proteomic analysis reveal that EBOV is composed of only seven genes encoding for eight proteins. The seven genes are for the nucleoprotein (NP), RNA dependent RNA polymerase L, glycoprotein (GP) and the viral proteins VP24, VP30, VP35, VP40 [3] EBOV-GP is expressed in two molecular forms viz., GP1 and GP2 which are held together by disulphide bond to form a heterodimeric protein. The EBOV entry in host cell is mediated by the viral spike

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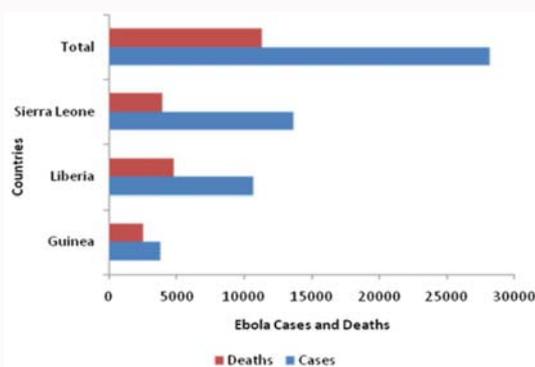


Figure 1: Ebola outbreak in Western Africa as of September, 2015.

Table 1: Proteins selected from Ebola virus for docking.

Name	PDB ID	Method	Resolution (Ao)	Role in ebola virus	Ref
Ebola glycoprotein-GP1	Modelled	Easy modeller 4.0	-	Host cell attachment and base for GP2	[10]
Ebola glycoprotein-GP2 (epo 74)	2EBO	X ray diffraction	1.9	Membrane fusion	[11]
Niemann-Pick C1(NPC-1)	3GKI 3GKJ 3GKH 3QNT	X ray diffraction	1.8 1.6 1.81 2.83	Escape from host immune system and binding site for viral infection	[12]

Table 2: Seventeen naturally existing compounds used for docking analysis.

Plant name	Family	Active components	PubChem ID	Medicinal uses	References
<i>Andrographis paniculata</i>	Acanthaceae	14-acetylandrographolide, 14-Deoxyandrographoside, Andrograpanin, Andrographolide, Isoandrographolide, Neoandrographolide	73353957, 133868, 11666871, 5318517, 101563021, 9848024	Upper Respiratory Infection, Ulcerative Colitis and Rheumatic Symptoms	[13-14]
<i>Curcuma longa</i>	Zingiberaceae	Curcumin	969516	Antifungal, antibacterial, kidney and cardiovascular diseases, arthritis	[15-16]
<i>Fumaria indica</i>	Papaveraceae	Fumaric acid, Monomethyl Fumarate	444972, 21721168	Aches, Pains, Diarrhoeas, Fever, Influenza, Liver Complaints	[17]
<i>Alhagi camelorum</i>	Fabaceae	Gallic acid, Rutin	370, 5280805	Gastroprotective, Diaphoretic, Diuretic, Expectorant, Laxative, Antidiarrhoeal and Antisepticpropertie, Rheumatism and Hemorrhoids	[18]
<i>Adhatoda vasica</i>	Acanthaceae	Vasicine, Vasicoline	667496, 626005	Anti Rheumatic, Anthelmintic, Sedative, Diarrhea, Dysentery, Antihemorrhagic and Antidiabetic	[19]
<i>Picrorhiza kurroa</i>	Plantaginaceae	Kutkoside, Apocyanin	182265, 2214	Antibacterial, antiperiodic, laxative, hepatoprotective, anticholestatic, anti-inflammatory, anti-allergy and antioxidant	[20]
<i>Azadirachta indica</i>	Meliaceae	Azadirachtin, Nimbin	5281303, 108058	Anthelmintic, Antifungal, Antidiabetic, Antibacterial, Antiviral, Contraceptive and Sedative	[21]

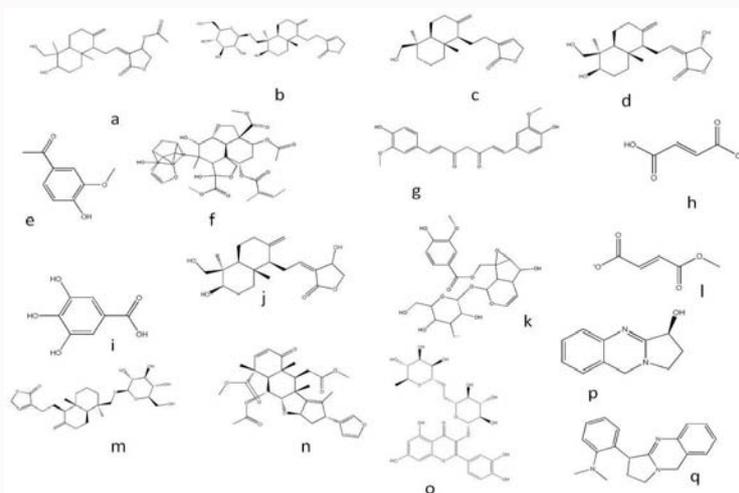


Figure 2: Selected ligand structure for analysing anti- Ebola activity using in silico methods. (a) 14-acetylandrographolide (CID: 73353957), (b) 14-deoxyandrographoside (CID: 133868), (c) andrograpanin (CID: 11666871), (d) andrographolide (CID: 5318517), (e) apocynin (CID: 2214), (f) azadirachtin (CID: 5281303), (g) curcumin (CID: 969516), (h) fumaric acid (CID: 444972), (i) gallic acid (CID: 370), (j) isoandrographolide (CID: 101563021), (k) kutkoside (CID: 182265), (l) monomethyl fumarate (CID: 21721168), (m) neoandrographolide (CID: 9848024), (n) nimbin (CID: 108058), (o) rutin (CID: 5280805), (p) vasicine (CID: 667496), (q) vasicoline (CID: 626005).

*CID: PubChem Compound Identifier

protein GP followed by cathepsin B and L digestion to release GP2 (in the endosomal compartments) [4].

Additional host factors are required for EBOV entry and release. For example, the Niemann-Pick C1 (NPC1) protein which mediates

intracellular cholesterol traffics to post-lysosomal destinations. The trimmed GP1 exposes the N-terminal domain that binds to the NPC1 followed by stimulation of fusion activity of GP2. Ebola and Marburg filovirus's infection requires NPC1 function, cells defective in NPC1 function and primary fibroblasts derived from human Niemann-Pick

type C1 disease patients were shown to be resistant to infection by the viruses. Small molecules such as U18666A and the antidepressant imipramine are known to target NPC1 and the resultant cells possess phenotype similar to NPC1 deficient ones. U18666A was found to inhibit EBOV infection at entry stage rather than replication, thus emphasizing the critical role of NPC1 in filoviral infection [5]. Development of GP antagonist as an antiviral therapy is also an attractive strategy with numerous reported studies. In one of the study, a chemical library of G protein - coupled receptor (GPCR) antagonists (targeting 5-HT (serotonin) receptor) were shown to block the GP mediated viral entry [6]. Structure based drug designing and docking studies have been carried to screen for anti-Ebola drugs through multiple targets. Flavonoids such as Gossypetin and Taxifolin have multitarget affinity against four Ebola viral receptors namely VP40, VP35, VP24 and VP30 [7]. Similarly by virtual screening techniques, four chemicals were found to bind to VP40 subunit by interfering sterically and preventing matrix protein oligomerization [8]. To date, there is no effective therapeutics available for the prevention or treatment of Ebola infections. Ayurvedic extracts and phytochemicals isolated from them are potential sources for novel anti-viral drugs based on different *in vitro* and *in vivo* approaches [9]. In an effort to screen for anti-Ebola agents, we selected EBOV-GPs and NPC1 as drug targets, due to their critical role in EBOV biology and analyzed seventeen active compounds from Ayurvedic plants through molecular docking techniques [10].

Materials and Methods

Protein preparation

Acquiring the three dimensional protein structures is a prerequisite for *in silico* docking analysis. In this study, we obtained three dimensional structures of Ebola viral proteins namely GP1, GP2 and NPC1. GP1 was modelled using Easy modeller 4.0 and others were obtained from PDB website (<http://www.rcsb.org/pdb/home/home.do>). Details about Ebola viral proteins selected for the study and their characteristic features in viral infections are represented in (Table 1). GP1 was modelled using Easy modeller 4.0 [11]. Initially, GP1 protein sequence as FASTA was retrieved from UNIPROT database [12] and the template was selected by querying sequence against PDB database using NCBI-BlastP program [13]. Chain I, Crystal Structure of the Trimeric Prefusion Ebola Virus Glycoprotein (PDB id: 3CSY I) was considered as template for homology modelling since the query sequence has 99% sequence identity with *e-value* of 0.0. The modelled structure was validated for its nature using Rampage server [14].

Ligand preparation

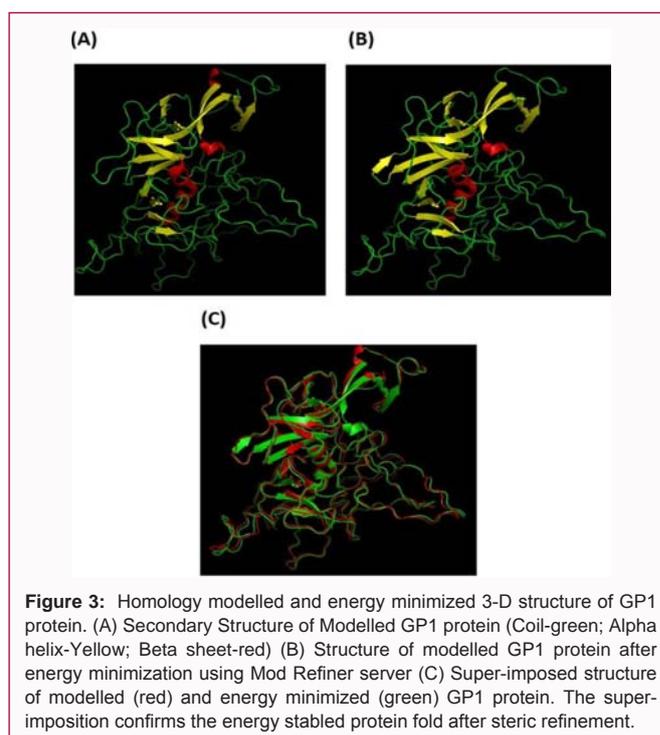
Seventeen compounds were chosen for the study from various medicinal plants showing anti-viral properties based on the recent literatures (Figure 2 and Table 2). The isomeric smiles of the selected ligands were obtained from pubchem database and their three dimensional structures were generated by CORINA online server [15] and saved as pdb files.

Molecular descriptors calculation

Molinspiration online database was used to calculate the selective descriptors to analyse the ligand properties [16-18]. The ligands were analysed for their logP, polar surface area, molecular weight, number of atoms, number of O or N, number of OH or NH, drug likeness values based on Lipinski's rule of five.

Molecular docking studies

Binding analysis of EBOV- GPs with individual ligands was



studied by docking software Auto dock 4.0. Auto dock 4.0 uses Monte Carlo simulated annealing and Lamarckian Genetic Algorithm (LGA) to create a set of possible conformations. LGA is used as a global optimizer and energy minimization as a local search method. Possible orientations are evaluated with AMBER force field model in conjunction with free energy scoring functions and a large set of protein-ligand complexes with known protein-ligand constant [19]. The newest version 4 contains side chain flexibility. Hydrogen atoms, Kollman charge. The grid was centred in the active site region which involves all functional amino acid residues. The active sites of all the proteins were identified by detecting larger volume of pockets using CASTp server, [20]. Grid maps were generated using the Auto grid Program. Docking was performed using the Lamarckian genetic algorithm. In the present study docking was performed by creating an initial population of 150 individuals, 5 random torsions to each of the 150 individuals, Lamarckian Genetic Algorithm (LGA), was implemented with a maximum of 2,500,000 energy evaluations.

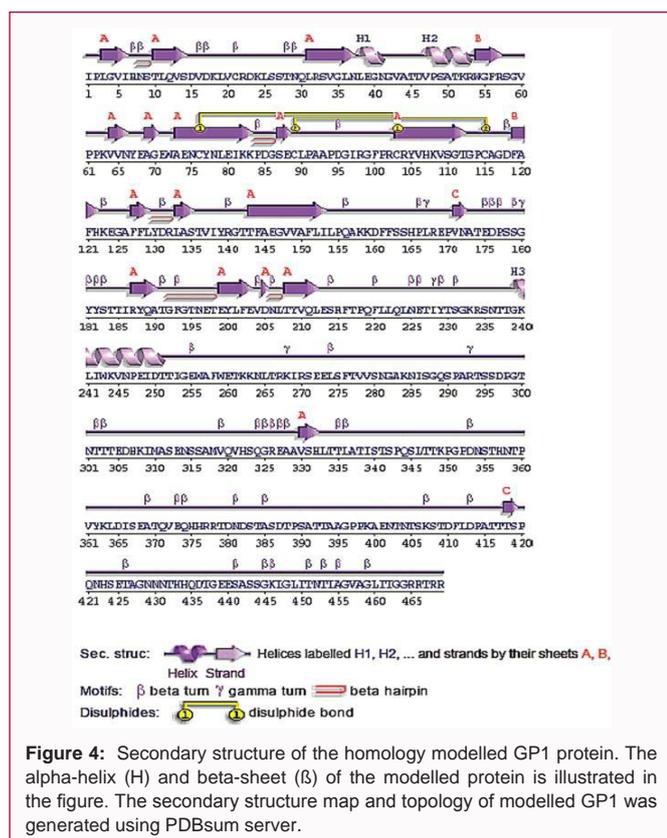
Results and Discussion

Homology modelling of GP1

The three dimensional structure of EBOV-GP1 was determined by homology modelling with crystal structure of the Trimeric Prefusion Ebola Virus Glycoprotein Chain-I (PDB id: 3CSY I) as template (see Materials and Methods). After structural validation with RAMPAGE server, it was observed that the amino acids (Asp160, Ala157, Thr174, Ser178, Phe161, Asp176) were sterically disallowed from the secondary structure. To improve the stability of protein, it was energy minimized using ModRefiner server [21] (Figure 3) shows the homology modelled GP1, energy minimized 3-D structure of GP1 protein and Super-imposed structure of modelled and energy minimized GP1 protein. The super-imposition confirms the energy stabled protein fold after steric refinement. The quality and reliability of the modelled GP1 was inspected by checking the backbone and side-chain conformations, bond lengths, angles, and residue contacts using ProCheck, [22] the results were within the reliable criteria (data

Table 3: Lipinski's rule of five drug-likeness properties of potential compounds for the selected ligands.

Ligand Name	LogP	TPSA	Natoms	Molecular weight	nON	nOHNH
14_acetylandrographolide	1.76	93.1	28	392.5	6	2
14-deoxyandrographoside	0.03	145.9	35	486.6	9	5
Andrograpanin	2.87	46.5	23	318.5	3	1
Andrographolide	1.05	87	25	350.5	5	3
Apocynin	1.18	46.5	12	166.2	3	1
Azadirachtin	1.42	215.4	51	720.7	16	3
Curcumin	2.3	93.1	27	368.4	6	2
Fumaric acid	-0.68	74.6	8	116.1	4	2
Gallic acid	0.59	98	12	170.1	5	4
Isoandrographolide	1.05	87	25	350.5	5	3
Kutkoside	-1.05	197.1	36	512.5	13	6
Monomethyl fumarate	-2.78	66.4	9	129.1	4	0
Neoandrographolide	1.17	125.7	34	480.6	8	4
Nimbin	3.55	118.4	39	540.6	9	0
Rutin	-1.06	269.4	43	610.5	16	10
Vasicin	1.04	35.8	14	188.2	3	1
Vasicoline	3.32	18.8	22	291.4	3	0



not shown). Further, the secondary structure of the modelled GP1 was generated using PDBsum server [23]. The alpha-helix and beta-sheet connected with coil clearly shows the topological arrangement 469 amino acid residues of modelled GP1 protein (Figure 4). The model was almost as good quality as those of the reference templates as evident from the results obtained using Ramachandran plot analysis (Figure 1) for comparison of stereo chemical and energetic properties of the models with those of the templates.

Nature of the ligands

The small molecule selected for the drug discovery programmes should be evaluated before the docking analysis. The Leads should be inspected for their drug likelihood for their bioavailability nature using the "Rule of 5". Lipinski rule of five, states that the better drug leads may have more than five hydrogen bond donors and less than ten hydrogen bond acceptors, a molecular weight less than 500, and a calculated log of the partition coefficient (clogP) less than 5. In addition, Veber and colleagues [18] stated that the rotatable bonds in the leads should be less than ten for higher bioavailability potential for oral medicines. The structure of the selected compounds for the present study was downloaded from the PubChem compound database of NCBI are shown in (Figure 2) and respective three dimensional structures were obtained using CORINA server. The descriptors of these compounds had been shown in (Table 3). Except azadirachtin, nimbin, kuthkoside and rutin, the rests of the compounds had molecular weight lesser than 500. Azadirachtin, rutin and kuthkoside had 16, 16 and 13 hydrogen donors respectively, but due to their usage in the traditional medicine, they were included in the study. The logP value of 14_acetylandrographolide, 14-deoxyandrographoside, fumaric acid was lesser than 1, but, their bioactive nature had been well established previously. Except them, other compounds have moderate to better log P values, indicating that they might be readily soluble in blood. The Topological Polar Surface Area (TPSA) of all compounds reveals them as good human intestinal absorbents.

Docking analysis

The entry of viruses into target host cells is the most attractive drug targets in viral therapies. The entry of EBOV into host cells requires the cooperating roles of viral genes such as NP, VP35, VP40, GP, VP30, VP24, L and other host proteins especially NPC1 [24]. The GP1 subunit is mainly responsible host cell recognition and attachment due to presence of glycosylated region termed the mucin-like a receptor- binding domain. GP1 is further sub divided into base, head and glycan cap. The GP1 base subdomain contains

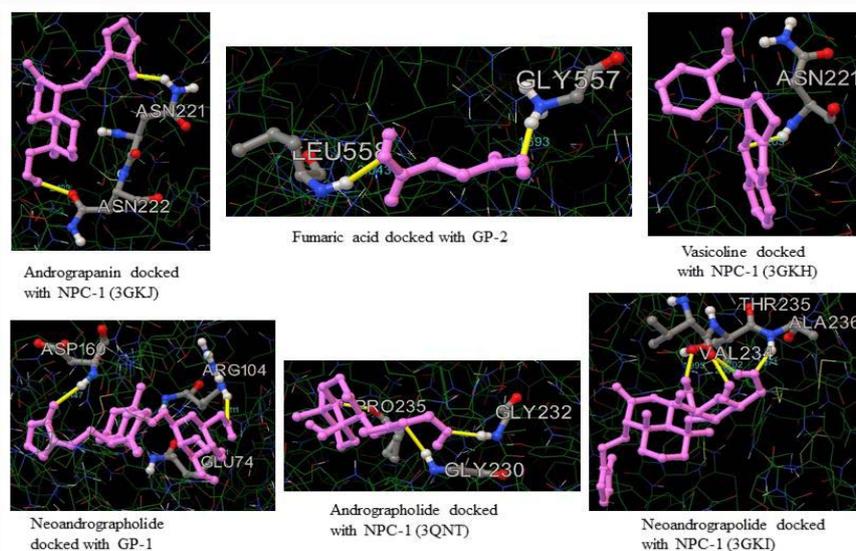


Figure 5: Proteins structures of the drug targets for the present study as viewed by PyMol. Out of seventeen ligands analysed for the study, six were shown strong hydrogen bonds with active site residues of the respective proteins. The active site residues had been highlighted in the figures.

Table 4: Best docking confirmations of the ligands against EBOV – GPs and Host cell proteins (different conformant of NCP1).

Protein Name	Ligand name	Amino acids involved in Hydrogen bond formation	Bond length(nm)
Ebola glycoprotein-GP1 (Designed in modeller)	Neoandrographolide	ARG - 104	2.111
		ASP - 160	2.147
		GLU - 74	2.110
Ebola glycoprotein-GP2 (Pdb id: 2EBO)	Fumaric acid	LEU - 558	1.741
		GLY - 557	1.701
Niemann-Pick C1(NPC-1) (Pdb id: 3GKI)	Neoandrographolide	ALA - 236	1.871
		VAL - 234	2.202
		VAL - 234	2.109
		THR - 235	1.995
		ASN - 221	2.033
Niemann-Pick C1(NPC-1) (Pdb id: 3GKJ)	Andrograpanin	ASN - 222	2.193
		ASN - 221	2.203
Niemann-Pick C1(NPC-1) (Pdb id: 3QNT)	Andrographolide	GLY - 230	2.196
		GLY - 232	1.900
		PRO - 235	2.205
		GLY - 230	2.196

four discontinuous sections (residues 33–69, 95–104, 158–167 and 176–189), and forms a hydrophobic, semicircular surface that interacts with heptad repeat region of GP2 for the attachment and stabilization of the infection. Further, this GP1 and GP2 complex, increase the possibility of the infection and lysis of the lysosome, by which the virion particles are dispersed for further infections. The present docking analysis revealed that neo andrographolide made a hydrogen bond with Arg 104 and Asp 160, GP1 base sub domain residues and thus may inhibit the normal GP1–GP2 complex. Hydrogen bonds and hydrophobic-contacts are the most important type of interactions to inhibit the native proteins. (Figure 5) illustrate the interaction of selected compounds with the proteins chosen for the study. Polar amino acid residues i.e. Arg104, Asp160 and Glu74 of P1 had strong H-bonding with the acetate group of the ligand. Neoandrographolide made hydrogen bonds with active site residues of the proteins irrespective of their polar nature (Table 4). This also showed the strongest binding affinity with proteins as incidental by its lowest internal energy (-3 to -8 kcal/mol), values are given in the (Table 5). In one of the earlier study, glutamic acid (E74) was targeted by alanine-scanning mutagenesis which resulted in a defect in virion incorporation. These mutants (E74A) were defective in GP incorporation into virions with loss of infectivity (Relative infectivity of 2%) [25]. Similarly the antibody, 16F6 that neutralises Sudan virus

through multiple viruses including G557 was found to bind to GP1-GP2 epitope [26]. EBOV GP2 contains two heptad repeat regions (HR1 and HR2), where the well-ordered HR1 region is subdivided into four segments (HR1_A-HR1_D) which assemble and encircle GP1 and promote lipid fusion and viral bursting [4] In our study, the fumaric acid hydrogen bonded with Leu 558 and Gly 557 (Table 4) respectively on the HR1_A and HR1_B; part of the GP2 heptate region which may inhibit the actual conformational change and inhibit the viral population bursting. Effects of inhibitors targeting these regions are yet to be studied in detail. The neoandrographolide, vasicoline and andrographolide were docked to the active sites of different conformant of NPC1 proteins. Those ligands formed stronger hydrogens bonds with the active sites (Table 4). In contrast, andrographolide solely bound to non – polar residues (Gly 230, 230 and Pro 235 respectively) of NCP1 for the inhibiting activity. The water molecules were eliminated in the docking analysis to explore the additional docking pose without excluding the possibility of direct hydrogen bonding. Neoandrographolide, fumaric acid, vasicoline and andrographolide were showed to inhibit the viral proteins with higher binding energy. The present study confirmed that the active molecules of the respective medicinal plants *viz.* *A. paniculata*, *F. indica* and *A. vasica* might inhibit the viral pathogenesis at various levels spanning from prevention to cure. Most of the traditional

Table 5: Auto Dock estimated free energies of binding (G) of phytochemicals in the active site of selected target proteins.

Target Protein	Ligand	Binding energy	Inhibition constant	Intermolecular energy	Ref RMS
Ebola glycoprotein-GP1 (Designed in modeller)	Neoandrographolide	-7.86	1.73	-11.14	59.38
Ebola glycoprotein-GP2 (Pdb id: 2EBO)	Fumaric acid	-6.89	8.89	-8.08	28.12
Niemann-Pick C1(NPC-1) (Pdb id: 3GKI)	Neoandrographolide	-6.9	8.71	-10.18	54.41
Niemann-Pick C1(NPC-1) (Pdb id: 3GKJ)	Andrograpanin	-6.71	11.99	-8.21	47.03
Niemann-Pick C1(NPC-1) (Pdb id: 3GKH)	Vasicoline	-6.77	10.97	-7.36	45.42
Niemann-Pick C1(NPC-1) (Pdb id: 3QNT)	Andrographolide	-6.69	12.49	-7.29	40.42

formulations have multiple medicinal plants as active ingredients; this study confirmed that the traditional formulation including active components from antiviral plants may be useful in prevention and elimination of the EBOV infection.

Conclusion

In silico based drug design is one of the potential techniques, especially when discovering new drug leads against essential drug targets. In this study, a preliminary assessment of the natural compounds based on their bioavailability related criteria, were docked with potential drug targets of ebola virus including GP1, GP2 and the host protein NPC1. Out of the seventeen leads, five showed prominent binding in active sites of the screened proteins. This study further confirmed that the ligands should be evaluated at laboratory level to fish out the anti-Ebola molecules. Neoandrographolide, andrograpanin, fumaric acid, vasicoline and andrographolide were shown to inhibit all the proteins selected in the study with higher binding energy. Since, the natural compounds show minimal side-effects comparatively with the synthetic ones, the use of these formulations through proper delivery platform will upgrade the single-dose regimens during outbreak and post-exposure scenarios.

Acknowledgments

This section should come before the References. Funding information may also be included here.

Appendix A. Appendices

Appendices should be used only when absolutely necessary.

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