Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [9]:12-23
[http://scholarsresearchlibrary.com/archive.html]

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

GP and NPC1 herbal targeted compounds in drug discovery and development – An *in silico* evidence for Ebola drugs

R Logesh R[1*], Parameswaran M[1], Dhanabal SP[1], Duraiswamy B[1], Dhamodaran P[1], Rajan S[2]

[1]*Department of Pharmacognosy and Phytopharmacy (Off campus, Jagadguru Sri Shivarathreeswara University), JSS College of Pharmacy, Rockland's, Ooty, India.*

[2]*Field Botanist, Survey of Medicinal Plants and Collection Unit, CCRH, Ministry of AYUSH, Ooty-643209, TN, India.*

***Corresponding author:** Logesh R, Department of Pharmacognosy and Phytopharmacy (Off campus, Jagadguru Sri Shivarathreeswara University), JSS College of Pharmacy, Rockland's, Ooty, India. Tel:+91-9488134810; E-Mail: rlogesh14@gmail.com.*

ABSTRACT

In silico based drug design is one of the most potential techniques in the discovery of new drug leads against essential drug targets. Ebola viruses (EBOV) of Filoviruses, consists of five species, viz. Zaire, Sudan, Ivory Coast, Bundibugyo, and Ravn, which acts by 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 mainly depends on viral recognition, attachment and transmission of virion to host cell and lysis. Recent research reveals that apart from 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 (Ayurvedic plants) are carried out based on bioavailability criteria, and 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 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, 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.

Key words: Ebola, ayurveda, glycoprotein, NPC1, andrographalide, docking, drugs, *In silico*

INTRODUCTION

The most complex Ebola outbreak of 2014 caused widespread pathogenesis and spreading 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 (WHO, 2015, Figure 1). Ebola belongs to the virus family of Filoviradae and five species have been identified: Zaire, Bundibugyo, Sudan, Reston and TaÏ forest. Zaire species has been responsible for the 2014 West African outbreak [1].

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 [2,3]. 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 protein GP followed by cathepsin B and L digestion to release GP2 (in the endosomal compartments) [4]. Additional host factors are required for EBOV to entry and release. For example, the Niemann-Pick C1 (NPC1) protein which mediates a intracellular cholesterol trafficking 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 [5]. Ebola and Marburg filoviruses infection requires NPC1 function protein, in which cells are 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. U18666A was found to inhibit EBOV infection at early entry stage rather than replication, thus emphasising the critical role of NPC1 in filoviral infection [5].

Development of GP antagonist as an antifiloviral therapy is also an effective strategy with wide 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 involving docking studies have been carried to screen for anti-ebola drugs through multiple targets rather than a single target. 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 stearically and preventing matrix protein oligomerization [8].

To date, there are 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 [10-15].

MATERIALS AND METHODS

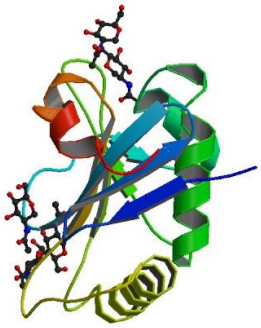
Protein preparation

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 is represented in (Table 1).

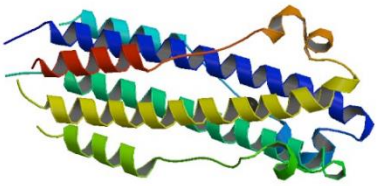
Table 1: Proteins selected from Ebola virus for docking.

Name	**PDB ID**	**Method**	**Resolution (A^o)**	**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	X ray diffraction	1.8	Escape from host immune system and binding site for viral infection	[12]
	3GKJ	X ray diffraction	1.6		
	3GKH	X ray diffraction	1.81		
	3QNT	X ray diffraction	2.83		

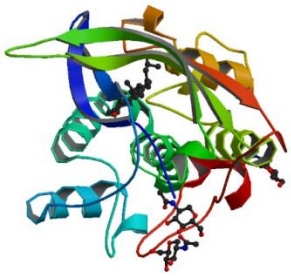
GP1 was modelled using Easy modeller 4.0, where four sequences were selected based on their highest sequence similarity (PDB id was 3CSY I, 2Y6S P, 2QHR P, 3S88 I). The modelled structure was validated for its nature using Rampage server [15-17].



GP1



GP2



NPC1

Figure 1: Ebola belongs to the virus family of Filoviradae and five species

Ligand preparation

Seventeen compounds were chosen for the study from various medicinal plants showing anti-viral properties based on the current literatures (Figure 2, 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 and saved as pdb files [18-21].

Molecular descriptors calculation

Molinspiration online database was used to calculate the selective descriptors to analyse the ligand properties [22-24]. 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 Autodock 4.0. Autodock 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 constants [25]. The newest version 4 contains side chain flexibility, hydrogen atoms, Kollman charges. The grid was centered in the active site region which involves all functional amino acid residues. Grid maps were generated using the Autogrid 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 2500000 energy evaluations [26,27].

Results and Discussion

Homology modelling of GP1

The three dimensional structure of EBOV–GP1 was determined by homology modelling with appropriate constraints based on the protein templates elucidated with X-ray crystallography. The selected templates comparatively displayed good similarities to the modelled protein as e-values equal to 0. 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, the results were within the reliable criteria (data not shown). The model was almost as good quality as those of the reference templates as evident from the results obtained using Ramachandran plot analysis (Sup Table. 1) for comparison of stereochemical 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 likeness 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 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 (Table. 2) and respective of their three dimensional structures were obtained using corina (online server). The descriptors of these compounds had been shown in Table 3. Except azadirachtin, nimbin, kuthkoside and rutin, the compounds had a molecular weight lesser than 500. Azadirachtin, rutin and kutkoside 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.

Table 2: Seventeen naturally existing compounds were selected based on the literature and used for docking analysis.

Plant name	Family	Vernacular name	Active components	Medicinal uses	References
Andrographis paniculata	Acanthaceae	Nilavembu	14-acetylandrographolide, 14-Deoxyandrographoside, Andrograpanin, Andrographolide, Isoandrographolide, Neoandrographolide	Upper Respiratory Infection, Ulcerative Colitis and Rheumatic Symptoms	[13-14]
Curcuma longa	Zingiberaceae	Manjal	Curcumin	Antifungal, antibacterial, kidney and cardiovascular diseases, arthritis	[15-16]
Fumaria indica	Papaveraceae	Pitpapra	Fumaric acid, Monomethyl Fumarate	Aches, Pains, Diarrhoeas, Fever, Influenza, Liver Complaints	[17]
Alhagi camelorum	Fabaceae	Javasa	Gallic acid, Rutin	Gastroprotective,Diaphoretic, Diuretic, Expectorant, Laxative, Antidiarrhoeal and Antisepticpropertie, Rheumatism and Hemorrhoids	[18]
Adhatoda vasica	Acanthaceae	Vasambu	Vasicine, Vasicoline	Anti Rheumatic, Anthelmintic, Sedative, Diarrhea, Dysentery, Antihemorrhagic and Antidiabetic	[19]
Picrorhiza kurroa	Plantaginaceae	Katukarogini	Kutkoside, Apocyanin	Antibacterial, antiperiodic, laxative, hepatoprotective, anticholestatic, anti-inflammatory, anti-allergy and antioxidant	[20]
Azadirachta indica	Meliaceae	Veppai	Azadirachtin, Nimbin	Anthelmintic, Antifungal, Antidiabetic	[21]

				, Antibacterial, Antiviral , Contraceptive andSedative	

Table 3: Lipinski's rule of five drug-likeness properties of potential compounds by using Molinspiration *web*-server for the selected ligands

Ligand Name	LogP	TPSA	natoms	Molecular weight	nON	nOHNH
14_acetylandrographolide	1.76	93.1	28.0	392.5	6	2
14-deoxyandrographoside	0.03	145.9	35.0	486.6	9	5
Andrograpanin	2.87	46.5	23.0	318.5	3	1
Andrographolide	1.05	87.0	25.0	350.5	5	3
Apocynin	1.18	46.5	12.0	166.2	3	1
Azadirachtin	1.42	215.4	51.0	720.7	16	3
Curcumin	2.30	93.1	27.0	368.4	6	2
Fumaric acid	-0.68	74.6	8.0	116.1	4	2
Gallic acid	0.59	98.0	12.0	170.1	5	4
Isoandrographolide	1.05	87.0	25.0	350.5	5	3
Kutkoside	-1.05	197.1	36.0	512.5	13	6
Monomethyl fumarate	-2.78	66.4	9.0	129.1	4	0
Neoandrographolide	1.17	125.7	34.0	480.6	8	4
Nimbin	3.55	118.4	39.0	540.6	9	0
Rutin	-1.06	269.4	43.0	610.5	16	10
Vasicin	1.04	35.8	14.0	188.2	3	1
Vasicoline	3.32	18.8	22.0	291.4	3	0

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 and other host proteins especially NPC1. 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 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 displayed 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. Neoandhrographlide 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%) (26). Similarly the antibody, 16F6 that neutralises Sudan virus through multiple viruses including G557 was found to bind to GP1-GP2 epitope (27).

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 fumeric 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. Effect of inhibitors targeting these regions are yet to be studied in detail.

Table 4: AutoDock estimated free energies of binding (G) of phytochemicals in the active site of selected target proteins

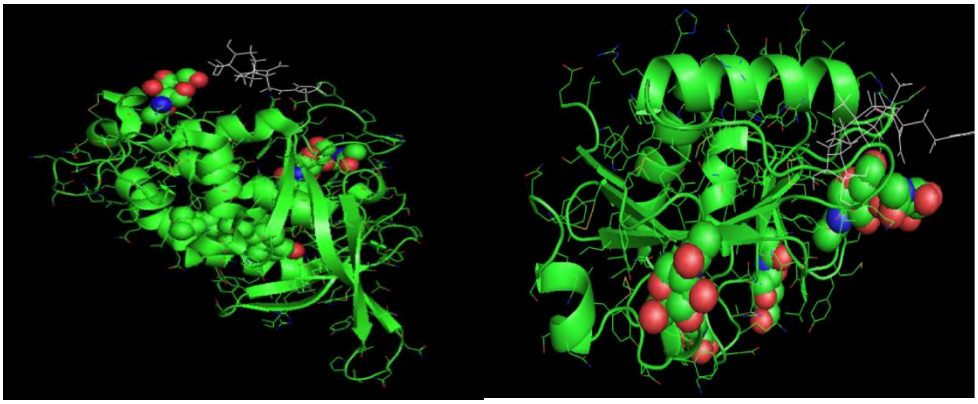
Target Protein	Ligand	Binding energy	Inhibition constant	Intermolecular energy	Ref RMS
Ebola glycoprotein-GP1 (Designed in modeller)	Neoandrographolide	-7.86	1.73	-11.14	59.38
Ebola glycoprotein-GP2 (Pdb id: 2EBO)	Fumaric acid	-6.89	8.89	-8.08	28.12
Niemann-Pick C1(NPC-1) (Pdb id: 3GKI)	Neoandrographolide	-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:	Vasicoline	-6.77	10.97	-7.36	45.42

3GKH)					
Niemann-Pick C1(NPC-1) (Pdb id: 3QNT)	Andrographolide	-6.69	12.49	-7.29	40.42

Table 5: Best docking confirmations of the ligands against EBOV – GPs and Host cell proteins (different conformants 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	2.11
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
Niemann-Pick C1(NPC-1)	Andrograpanin	ASN - 221	2.033
		ASN - 222	2.193
Niemann-Pick C1(NPC-1)	Vasicoline	ASN – 221	2.203
Niemann-Pick C1(NPC-1)	Andrographolide	GLY - 230	2.196
		GLY - 232	1.9
		PRO - 235	2.205

The neoandrographolide, vasicoline and andrographolide were docked to the active sites of different conformants of NPC1 proteins. Those ligands formed stronger hydrogens bonds with the active sites (Table 5). 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.



Andrographolide binds with NPC-1 **Neo-andrographolide binds with GP1**

Figure 2: The isomeric smiles of the selected ligand

Neoandrographolide, fumaric acid, vasicoline and andrographolide was 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 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, the use of these formulations through proper delivery platform will upgrade the single-dose regimens during outbreak and post-exposure scenarios.

Acknowledgements

The authors are very much thankful to Dr. S.P. Dhanabal and Dr. B. Duraiswamy for their support and to carry out the work. The authors are also thankful to the Department of Pharmacognosy, J.S.S college of Pharmacy, Nilgiris, India for financial support. I am very thankful to Dr. S. Rajan, Centre of Medicinal Plants Research in Homeopathy, Ministry of AYUSH, Indra nagar, Emerald for his encouragement and helpful in the identification and authentication of the plants.

References

1. Baize, S., Pannetier, D., Oestereich, L., et al. Emergence of Zaire Ebola Virus Disease in Guinea. *New Engl J Med*, **2014.** 371:1418–25.
2. WHO. Ebola Situation Report - 9 September 2015. http://apps.who.int/ebola/current-situation/ebola-situation-report-9-september-2015.
3. Mühlberger, E., Filovirus replication and transcription. *Future virol*. **2007.** 2:205-215.
4. Lee, JE., Saphire, EO., Ebola virus glycoprotein structure and mechanism of entry. *Future virol*. **2009.** 4:621-635.
5. Carette, JE., et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature*. **2011.** 477:340-343.
6. Cheng, H., et al. Inhibition of Ebola and Marburg Virus Entry by G protein-Coupled Receptor Antagonists. *J Virol.* **2015.** 89: 9932-9938.
7. Raj, U., and Varadwaj, PK., Flavonoids as Multi-target Inhibitors for Proteins Associated with Ebola Virus: In Silico Discovery Using Virtual Screening and Molecular Docking Studies. *Interdiscip Sci Comput Life Sci*. **2015**. 7:1-10.
8. Abazari, D., et al. Molecular docking based screening of predicted potential inhibitors for VP40 from Ebola virus. *Bioinformation*. **2015.** 11:243-247.
9. Aggarwal BB, et al. Identification of Novel Anti-inflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases: "Reverse Pharmacology" and "Bedside to Bench" Approach. *Curr drug targets*. **2011.** 12:1595-1653.
10. Bagchi, P., Mahesh, M., and Somashekhar, R., Pharmaco-Informatics: Homology Modelling of the Target Protein (GP1, 2) for Ebola Hemorrhagic Fever and Predicting an Ayurvedic Remediation of the Disease. *J Proteomics Bioinform*. **2009.** 2: 287–294.
11. Vladimir, N., et al. Core structure of the envelope glycoprotein GP2 from Ebola virus at 1.9-A resolution. *Proc Natl Acad Sci* USA, **1999.** 96: 2662–2667.
12. Kwon, HJ., et al. Structure of N-terminal domain of NPC1 reveals distinct subdomains for binding and transfer of cholesterol. *Cell*. **2009.** 137: 1213–1224.
13. Wintachai, P., et al. Activity of andrographolide against chikungunyavirus infection. *Sci Rep*. **2015.** 18; 5:14179.
14. Hossain, MS., et al. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. *Scientific World Journal*. **2014.** 2014:274905.
15. Zorofchian, MS., et al. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *BioMed Research International*. **2014.** 2014:186864.

16. Araújo, CC., and Leon, LL., Biological activities of *Curcuma longa* L. *Mem Inst Oswaldo Cruz*, **2001.** 96:723-728.
17. Gupta, PC., Sharma, N., and Rao, CV., A review on ethnobotany, phytochemistry and pharmacology of *Fumaria indica* (Fumitory). *Asian Pacific Journal of Tropical Biomedicine*. **2012.** 2:665-669.
18. Muhammad, G., et al. Alhagi: a plant genus rich in bioactives for pharmaceuticals. *Phytother Res*. **2015.** 29(1):1-13.
19. Claeson, UP., et al. Adhatoda vasica: a critical review of ethnopharmacological and toxicological data. *J Ethnopharmacol*. **2000.** 72(1-2):1-20.
20. Simonyi, A., Serfozo, P., Lehmidi, TM., et al. The neuroprotective effects of apocynin. *Frontiers in bioscience (Elite edition)*. **2012.** 4:2183-2193.
21. Subapriya, R., and Nagini, S., Medicinal properties of neem leaves: a review. *Curr Med Chem Anticancer Agents*. **2005.** 5(2):149-6.
22. Ertl, P., Rohde, B., and Selzer, P., Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J Med Chem*. **2000.** 43: 3714–3717.
23. Lipinski, CA., et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delivery Rev*. **1997.** 23: 4–25.
24. Veber, DF., et al. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem*. **2000.** 45: 2615.
25. Morris, G., and Huey, R., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*. **2009.** 30: 2785–2791.
26. Manicassamy, B., et al. Comprehensive analysis of ebola virus GP1 in viral entry. *J Virol*. **2005.** 79:4793-805.
27. Bale, S., et al. Structural Basis for Differential Neutralization of Ebolaviruses. *Viruses*. **2012.** 4:447-470.