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MOLECULAR MODELING AND DOCKING ANALYSIS OF SWINEFLU (H1N1) NEURAMINIDASE PROTEIN AGAINST THE PHYTOCHEMICALS OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

Background: Swine flu has become a life-threatening disease involving two main proteins neuraminidase (NA) and hemagglutinin (HA). Inhibition of NA, the protein responsible for initiation of viral infection is the important treatment step in the eradication of swine flu**.** *In silico* analysis of natural compounds for swine flu treatment serves as a lead for drug development.
Objective: The aim of this study is to obtain the 3 dimensional structure of Swine flu (HIN1) virus NA protein and to perform docking analysis to identify major residues involved in the binding of agonist and antagonist.
Methods: H1N1 NA protein structure was predicted using the principles of homology modeling using Swiss model. The predicted protein structure was then subjected to docking studies with phytocompounds from *Andrographis paniculata* using Autodock 4.2 tools. A total of 16 compounds were tested for Lipinski's rule and molecules which satisfied Lipinski's rule were subjected to docking analysis against NA protein of H1N1.
Results: Homology modeling is widely used structure prediction method for the proteins with no known experimental structures. In this study, the structure of NA protein was predicted using Swiss model. The protein was energy minimized using Swiss PDB Viewer and the final energy of the protein obtained was -87624.516 KJ/Mol. The predicted model was then subjected to docking studies. The compounds showed a binding energy in the range of -2.31 kcal/mol to -8.61 kcal/mol. The control drug Ostelmivir showed a binding energy of -3.35 kcal/mol.
Conclusion: The structure of H1N1 NA was predicted using the principles of homology modeling. The docking results showed that andrograpanin and neoandrographolide were found to have binding energies of -8.61 kcal/mol and -7.39 kcal/mol respectively. These compounds showed good inhibitory effect by binding to the active site of the protein. The identified plant-based compounds can be an alternative to chemically synthesized drug in treating swine flu. Hence, these compounds can be further considered for *in vitro* and *in vivo* evaluation against swine flu virus.

Keywords: Autodock 4.2, Homology Modeling, Neuraminidase (NA), Swine Flu, Swiss Model

INTRODUCTION

H1N1 is a type of influenza virus that has a size of about 80–120 nanometers in diameter and roughly spherical **(Dandagi and Byahatti, S. M. 2011**). The envelope of the virus is made of proteins HA and NA. The nuclear material is single-stranded RNA with a size of about 13.5 kB **(Kuroda *et al.*, 2010**). The H1N1 influenza epidemic has resulted in 3 to 5 million serious cases of infection with around 250 000 and 500 000 deaths worldwide **(Purohit *et al.*, 2018)**. Acute disease symptoms include headache, cough, fever chills, sore throat rhinorrhea, redness and watery eyes, vomiting, diarrhea, dyspnea, tachypnea, myalgia, arthralgia **(Jilani *et al.*, 2020**). The symptoms persist for about two to seven days, and the disease is mostly self-limited in healthy individuals, but cough, discomfort can continues to exist for up to 2 weeks in some patients **(Penteado *et al.*,2018**). Patients with chronic lung diseases, cardiac disease, and pregnant ladies are at higher risk of severe complications **(Lippi *et al.*,2010; Lim, and Mahmood, 2011; Okur *et al.*, 2013**). In addition to H1N1 infection patients are also susceptible to viral and bacterial pneumonia, hemorrhagic bronchitis, and possibly death and can occur within 48 hours from the onset of acute symptoms. NA and HA are the major proteins involving in the entry of H1N1 virus into the host cell. The NA binds to Sialic acid on the cell surface triggers the activation of clathrin mediated endocytosis enabling the viral entry.Vaccines are developed for influenza but there is a development of mutant strains day to day making the vaccines to be updated every year **(Elumalai *et al.*,2016**).
Many medicinal plants are proved to have anti-viral activity and hence can be exploited for the development of antiviral drugs. *Andrographis paniculata* Burm.f. Nees, commonly known as Kalmeghand Nilavembu in tamil is used for many decades against common cold, fever and inflammation, etc. It is one among the major constituent of at least 26 ayurvedic formulations described by Indian Pharmacopoeia. A wide range of phytocompounds including wogonin, carvacrol, myristic acid, chlorogenic acid have been reported in this plant **(Rajasekaran *et al.*, 2016; Sharma and Sharma, 2013; Payal *et al.*, 2015; Hossain *et al.*, 2014; Kopp and Schwede, 2004**)

Prevention and treatment for swine flu still remains challenging and requires a serious hunt for better anti-viral drugs. Bioinformatics approaches can be used for studying the interaction of plant compounds with NA protein a major protein in pathogenesis of swine flu and this knowledge is essential to discover a potential new drug for H1N1. This study involves homology modeling of H1N1 NA protein and comparison of the binding energy of about 16 phytocompounds of plant *Andrographis paniculata* Burm.f. Nees against the positive control drug (Oseltamivir) with the NA viral proteins. Currently, plant based herbal medicine are of much interest in treatment of many diseases because of high efficacy and less side effects compared to synthetic drugs. For H1N1 NA many plant phytocompounds like
All the compounds chosen for docking studies were screened based on literature studies. Many *In silico, In vitro* and *In vivo* studies have shown that the phytocompounds selected for this study have good positive results in treatment of various diseases. *In silico* techniques such as docking studies is used to find the binding ability of the phytochemicals to the viral proteins and if these compounds selectively bind to specific targets than the control drug then they could potentially be used more broadly in H1N1 influenza prevention and treatment.

MATERIALS AND METHODS

NA of H1N1

NA protein of H1N1 was selected for this study. The protein sequence of H1N1 NA (ID: ADJ40637.1) was retrieved using NCBI (http://www.ncbi.nlm.nih.gov/)

Homology modeling and structural Validation of Swine Flu NA

Swiss-Model server was used for modeling of the tertiary structure of NA protein using PDB ID: 5NWE as the template. It is a free web-based server to predict the structure of a protein sequence using the principles of homology modeling. Structurally homologous proteins are chosen as the template for structure

prediction because a structure of a protein is conserved compared to the amino acids, hence a query sequence structure can be predicted with high accuracy using homology modeling using a sequence of known structure (template) (**Biasini *et al.*, 2014**). Energy minimization of the modeled protein was performed using Swiss PDB viewer (**Dammalli *et al.*, 2014; Laskowski *et al.*, 1993**).

Validation

The refined model reliability was evaluated through SAVES server: PROCHECK, verify 3D and PROVE. These validation methodologies show the quality of protein structure and gives scores based on the protein quality (**Eisenberg *et al.*,1997; Colovos and Yeates, 1993; Dundas *et al.*,2006**).

Active Site Identification

Active site determination was done for the modelled protein using CASTp to further work on its docking studies. Active site determination is important for preparing grid box before docking (**Chao and Lin, 2010**).

Preparation of protein and ligand structure

The final energy minimized protein was chosen for docking. Before docking studies, the protein was prepared by adding polar hydrogen atoms and kollman charges using Autodock 4.2. The active compounds from *Andrographis paniculata* were chosen for docking studies. A total of 16 test compounds: neoandrogrpholide, andographin, andrographolide, wogonin, stigmasterol, andropanoside, octadeconoic acid, beta sitosterol, 14-deoxyandrographolide, andrographiside, chlorogenic acid, cinnamic acid, andrograpanin, lupeol and apigenin were chosen. All these compounds are proved to have many medicinal benefits (**Tan*et al.*, 2016; Rigsby and Parker, 2016**). The structure of these ligands was downloaded from PubChem Database in the SDF format was first converted to the PDB format using Pymol (**Benet et al.., 2016; Hari, 2019**). The ligands and their SDF structures were given in Table1.

Table 1 Details of phytochemicals of *Andrographis paniculata*

S.No.	Compound	Molecular Formula
1	14-deoxyandrographolide	$C_{20}H_{30}O_4$
2	Andrographin	$C_{20}H_{30}O_5$
3	Andrograpanin	$C_{20}H_{30}O_3$
4	Andrographiside	$C_{26}H_{40}O_{10}$
5	Andrographolide	$C_{20}H_{30}O_5$
6	Andropanoside	$C_{26}H_{40}O_9$
7	Apigenin	$C_{15}H_{10}O_5$
8	Beta Sitosterol	$C_{29}H_{50}O$
9	Chlorogenic acid	$C_{16}H_{18}O_9$
10	Cinnamic acid	$C_9H_8O_2$
11	Eugenol	$C_{10}H_{12}O_2$
12	Lupeol	$C_{30}H_{50}O$
13	Neoandrogrpholide	$C_{26}H_{40}O_8$
14	Octadeconoic acid	$C_{18}H_{34}O_2$
15	Stigmasterol	$C_{29}H_{48}O$
16	Wogonin	$C_{16}H_{12}O_5$

Drug Scan

All the ligands were tested for Lipinski's rule of five parameters such as molecular weight, log P, and number of hydrogen bond donors and number of hydrogen bond acceptors using molinspiration server (**Adejoro *et al.*, 2016**). Molinspiration and Lipinski Filters were applied for knowing the druggability of ligands by analyzing the number of hydrogen bond acceptor, the amount of hydrogen bond donors, Log P, and the molecular mass of the drugs (**Morris *et al.*, 2009**).

Preparation of Protein Structure

Docking Analysis of Protein-Ligand

Grid box

AutoDock 4.2software was used to generate grid box based on the active site of the protein. The grid-box was created that was large enough to cover the entire protein binding site and accommodate all ligands to move freely in it. The number of grid points in x, y, and z axes were set to 40×40×40. The X, Y and Z coordinates were also adjusted based on the active site of the protein (**Vyas *et al.*, 2008**).

Ligand docking

AutoDock 4.2 was used for docking of ligands to the catalytic triad of protein**.** Binding potential and possible conformations of ligand binding to the NA protein binding site can be assessed by doxking studies. To perform docking, initially the protein was fixed by adding polar hydrogen atoms and Kollman charges. Lamarckian Genetic Algorithm (LGA) method was used for ligand flexible protein-fixed docking studies. The best conformation was chosen with the maximum hydrogen bondings. Standard docking settings were used, and the 10 energetically most favorable binding poses were outputted (**Rognan, 2011**).

RESULTS AND DISCUSSION

Modeling and validation of H1N1 NA protein

The structure of H1N1 NA protein was unavailable in Protein Data Bank and hence it was modeled using Swiss Model. The sequence of H1N1 NA protein was retrieved from NCBI with ID: ADJ40637.1. The structure building of protein was performed using Swiss Model. Swiss Model is a fully automated tool for protein structure prediction that works based on the principles of homology modeling. Homology modeling method of protein structure prediction is based on a homologous template with known 3D structure. The template 5NWE was chosen based on the BLAST similarity search with the template having a identity score of 99.23%. The template 5NWE is the mutated crystal structure of NA, which a homotetramer with one amino acid residue (ASN 146) interacting with the ligand2-Acetamido-2-Deoxy-Beta-D-Glucopyranose (NAG). The final model was saved and viewed under Pymol molecular viewer (Figure 1).

Figure 1 Structure of NA protein (ID: ADJ40637.1) obtained using SWISS MODEL and viewed using Pymol Viewer.

The obtained model was then energy minimized using Swiss PDB viewer and the final energy of the model was found to be -87624.516 KJ/Mol (Table 2).

Table 2-Swiss PDB Viewer results of energy minimization of H1N1 NA protein

Bonds (KJ/mol)	1313.567
Angles	5610.952
Torsion	8984.906
Improper	1283.059
Non-Bonded	-59622.59
Electrostatic (KJ/mol)	-45194.45
Total (E)	-87624.516

The predicted model was then validated using Verify-3D and PROCHECK Ramachandran plot. Ramachandran plot provides information about the dihedral angles like φ (phi), ψ (psi) and ω in a protein. The ProCheck analysis revealed that 88.3% amino acid were in the most favored region and no amino acids were in the disallowed region which was shown in Ramachandran plot (Table 3 and Figure 2).

Table 3- Results from PROCHECK

Residues in most favoured regions [A, B, L]	88.3%
Residues in additional allowed regions [a,b,l,p]	11.4%
Residues in generously allowed regions [~a,~b,~l,~p] 1	0.3%
Residues in disallowed regions	0.0%

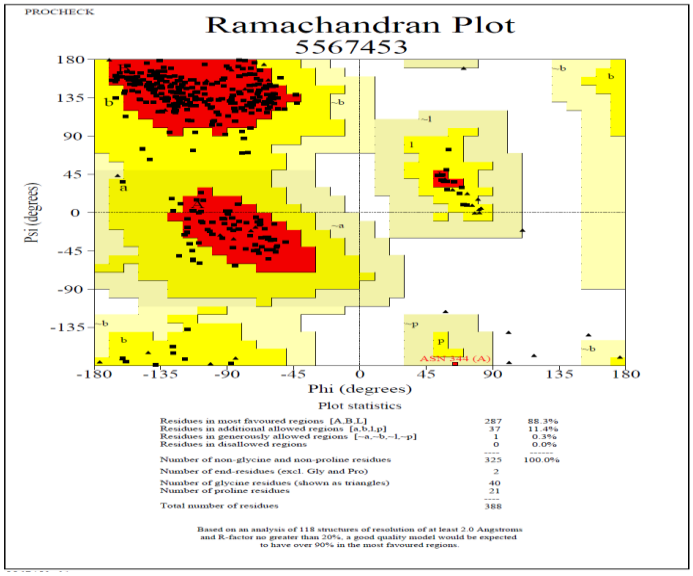


Figure 2 Ramachandran plot of predicted NA model (the red, dark yellow, and light-yellow regions represent the most favored, allowed, and generously allowed regions)

Verify-3D shows information on the compatibility between the 3D atomic model and the protein aminoacid sequence and compares the results to valid structures. According to verify 3D, a score above zero is considered good model. In this study, verify 3D provided a good compatible score with about 97.81% of the residues having averaged 3D-1D score >= 0.2, which was within the native confirmation of the crystals (Figure 3) and the profile score above zero indicated the acceptable environment of the model. In a similar modeling of lipase protein, models with average of 80% residues with verify 3D score >= 0.2 was considered a good model (**Sahoo *et al.*, 2019**).

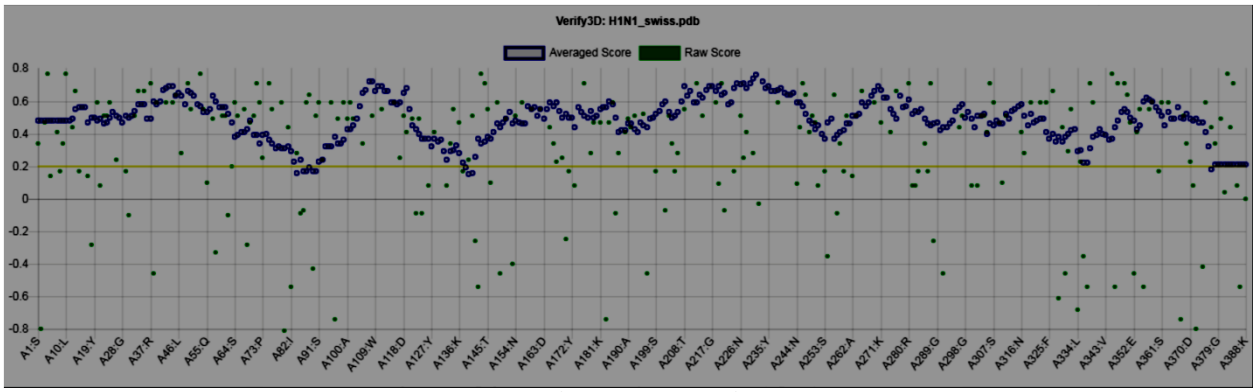


Figure 3- Verify 3D Z-score plot of HINI NA

The overall quality of the predicted model was analyzed using PROVE which showed satisfactory results of highly reliable model with compatible Z-score values (Figure 4). The modeled structure was highly similar to the crystal structure of the mutated NA protein that was available. However, in the crystal structure only one amino acid has been found to interact with the ligand, but the modeled structure most of the amino acids in active site has been found to interact with the ligands.

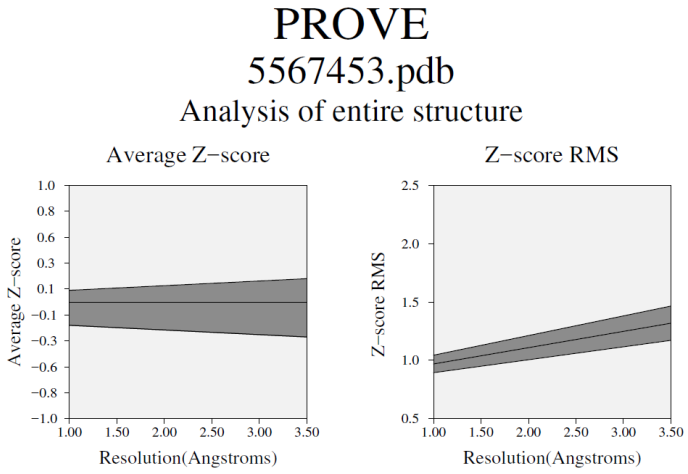


Figure 4- Results of PROVE showing Z-score values

Docking analysis of H1N1 NA protein with the bioactive compounds of *Andrographis paniculata.*

Natural compounds play an important role in reducing the virulence of an infection by minimizing the side effects of commercial drugs and many researches focuses on understanding the mechanism of interaction of these natural plant compounds with the drug targets of infectious diseases using *in silico* approaches (**Meenambiga *et al.*, 2018; Gupta *et al.*, 2013, Seniya *et al.*,2014**). Very few *in silico* studies have been performed with NA against natural inhibitors to recommend natural ligands as drugs targeting NA of Swine flu. In our present investigation, we have modeled the structure of NA to perform docking analysis against the bioactive compounds of *Andrographis paniculata*. Similar study was performed using Andrographolide, a major constituent of the plant *Andrographis paniculata* to identify its binding mechanism through virtual screening and molecular docking approaches (**Liu *et al.*, 2007**). The active site of the modelled protein was predicted using CASTp server and was shown in Table 4 and Figure 5. The total area and the volume of active site based on solvent-accessible surface was 187.500 and 152.523 respectively.

Table 4 Active site residues of modelled protein of H1N1 NA predicted using CASTp server.

Amino acid	Position	Amino acid	Position
ARG	118	THR	226
GLU	119	GLU	228
ASP	151	GLY	245
ARG	152	PRO	246
ARG	156	SER	247
TRP	179	GLU	277
SER	180	GLU	278
ASN	222	ARG	293
ILE	223	TYR	402
ARG	225		

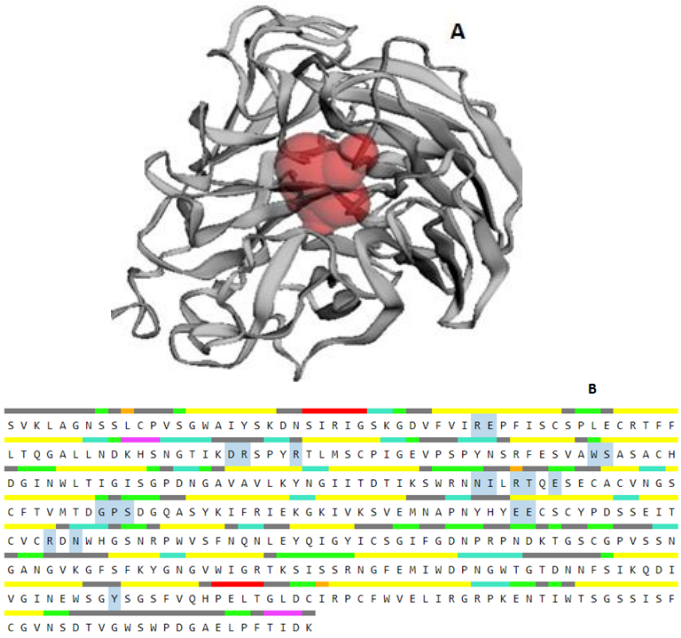


Figure 5 A) Active site residues of NA protein predicted using CASTp server. **B)** Letters highlighted in blue indicate the amino acids in the active site pocket of NA protein.

The details of about 16 compounds of *Andrographis paniculata* were obtained from PubMed literatures and those compunds were tested for their drug likeness property using Lipinski's rule of five. Among them, 10 compounds satisfied Lipinski's rule and they were further subjected to docking studies against H1N1 NA protein (Table 5).

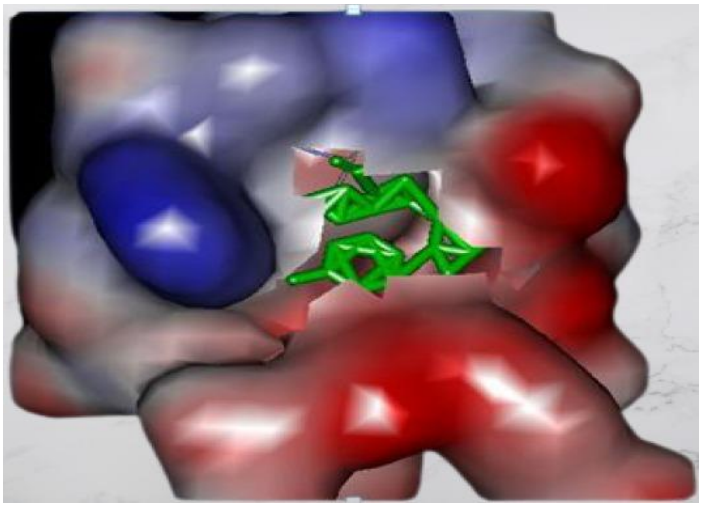


Figure 6- Docked confirmation of the compound Andrograpanin with NA

Table 6 lists the results of docking analysis of the ligands with NA protein. The andrograpanin compound has the highest binding energy with the active cavity of NA protein; the docked confirmation is given in Figure 6.

Table 5- Lipinski properties of bioactive compounds of *Andrographis peniculata*

S.No	Compound	Molecular weight (<500 Da)	Log P (<5)	H-bond donor (<5)	H-bond acceptor (<10)	Number of Violations
1	14-deoxyandrographolide	334.46	1.72	4	2	-
2	Andographin	328.32	3.27	1	6	-
3	Andrograpanin	318.46	2.87	1	3	-
4	Andrographiside	**512.6**	-0.66	**6**	10	3
5	Andrographolide	350.45	1.05	3	5	-
6	Andropanoside	496.6	0.02	5	9	-
7	Apigenin	270.24	2.46	3	5	-
8	Beta sitosterol	414.72	**8.62**	1	1	1
9	Chlorogenic acid	354.31	-0.45	**6**	9	1
10	Cinnamic acid	148.16	1.91	1	2	-
11	Eugenol	166.22	2.58	1	2	-
12	Lupeol	426.73	**8.29**	1	1	1
13	Neoandrogrpholide	246.22	1.7	5	3	-
14	Octadeconoic acid	284.48	**8.07**	1	1	1
15	Stigmasterol	412.7	**7.87**	1	1	1
16	Wogonin	284.27	2.96	2	5	-

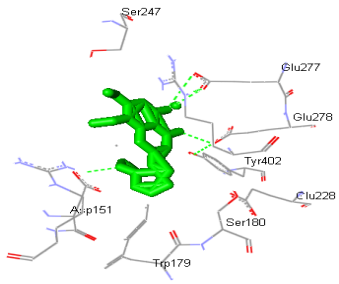
Symbol '-'indicates no violation

The docking analysis of the compounds with the modeled protein revealed that andrograpanin, a diterpene lactone isolated from *Andrographis peniculata* have the highest binding energy of -8.61 kcal/mol forming a hydrogen bond at Arg 152 (Figure 7a). Andrograpanin have already been proved for its antiinflammatory property in lipopolysaccharide induced macrophage cells. Neoandrographolide which is also a diterpene lactone possess a good binding energy of -7.39 kcal/Mol with the active site region of H1N1 NA receptor forming four hydrogen bonds at Arg 118, Arg 152, Glu 228 and Arg 368 (Figure 7b). The compound has good antiinflammatory property which has been studied by invivo methods (**Sahoo *et al.*, 2016**). The terpenoid compound andrographolide and its derivative 14-deoxyandrographolide have good binding energies of -4.38 kcal/Mol (Figure 7c) and -3.81 kcal/mol (Figure 7d) respectively. Both the compounds bind in the active site region of NA receptor forming hydrogen bonds at Arg 152 and Glu 288. Similar results were obtained when andrographolide was docked against Influenza A NA protein (**Seong *et al.*, 2018**). Further, compounds such as apigenin, wogonin, eugenol, andropanoside and andrographin were also found to inhibit NA of H1N1 with binding energy in the range of -5.59 kcal/mol to -4.12 kcal/mol (Figure 7e-7i) and the results were compared with the standard drug Ostelmivir (Figure 7j). The major constituents of the plant andropanoside and andrographin form hydrogen bond at the residues Arg 152, Glu 277 and Arg 293 which forms the active site pocket of NA protein. Wogonin, a flavonoid from *Scutellaria baicalensis* has anti-viral activities influenza virus in human lung epithelial cells (**Liu *et al.*, 2008**). Apigenin, an active flavonoid compound was proved for its highest antiviral activity against the infuenza virus (H3N2) (**Marchese *et al.*,2017**). Similary, eugenol showed the binding energy of -4.19 kcal/mol at the active site region of neuramindase. The compound eugenol has diverse biological activities such as antibacterial, antifungal, and antiviral properties (**Carrasco *et al.*,2012; Benencia and Courreges, 2000**). The standard compound Ostelmivir has a binding energy of -3.35 kcal/mol which was comparatively similar to our bioactive compounds used in this study. Thus, these compounds could effectively be used as leads for treating H1N1 infection. *In vitro* studies showed that many plants are potent inhibitor of NA of H1N1 like the *Rubia yunnanensis* root extract showed a maximum inhibition of 10%. The aqueous extract of whole plant of *Achyranthes aspera* showed an inhibition percentage of 43.67 again NA. *Carthamus tinctorius* flowers showed a maximum inhibition of 51%. Root extract of *Geranium strictipes* showed 85% inhibition against NA. The whole plant of *Balanophora involucrate* showed around 64% inhibition while the whole plant of *Euphorbia hirta* showed around 61% inhibition. The roots of *Paeonia delavayi* showed a maximum inhibition of about 92%. The root tuber of *Fagopyrum dibotrys* showed 70% inhibition, while root tubers of *Polygonum multiflorum* showed 78% inhibition. The roots of*Polygonum aubertii* showed an inhibition of around 86%. The rhizomes of*Curcuma longa* showed an inhibition percentage of around 77. This shows that many plant compounds are potent inhibitors and can be used to treat H1N1 disease **(Yang et al., 2016)**. From *In silico* studies, the

compounds from Andrographis paniculata showed good binding abilities, hence further *in vitro and in vivo* studies can be performed to confirm their effectiveness in targeting H1N1 NA.

Table 6- Molecular docking analysis of phytochemicals of *Andrographis paniculata* against H1N1 NA protein

S.No	Compound name	Binding energy (kcal/mol)	No. of H bonds	H bond interaction residues	Other interacting residues	No. of direct contacts (all polar, non-polar interactions)
1	14 deoxyandrographolide	-3.81	1	ARG152	ASP151, ARG156, ARG118, TRP129, GLU119, SER180, TYR402, ARG225, GLU278, THR226, GLU228	12
2	Andrograpanin	-8.61	1	ARG152	ILE223, ASP151, ARG156, TRP179, GLU119, SER180, TYR402, ARG225, GLU278, GLU228	11
3	Andrographin	-4.41	2	ARG152, GLU277	ILE223, SER247, ARG225, ASP151,TRP179, SER180, TYR402, GLU228, GLU278	11
4	Andrographolide	-4.38	2	ARG152, GLU 228	SER247, ILE223, ASP151, TRP179, SER180, GLU228, ARG225, GLU277, GLU278	11
5	Andropanoside	-5.59	2	ARG 152, ARG293	ILE223,ARG225,ASP151,TRP179,SER180,GLU278,ARG293,GLU228,GLU119, ARG118,TYR402,ARG368	14
6	Apigenin	-4.32	3	ARG 118, ARG 152, TRP 179	ARG293,GLU278,GLU228,ILE223,SER180,ASP151,GLU119,TYR402	11
7	Cinnamic acid	-2.31	1	SER 442	LYS150, ARG152, ASP151	4
8	Eugenol	-4.19	3	ARG118, ARG 293, ARG 368	-	
9	Neoandrogrpholide	-7.39	4	ARG 118, ARG 152,GLU 228, ARG368	TRP179,ASP151,SER180,TYR402,ARG 225,GLU278,GLU277,ARG293,THR226	12
10	Ostelmivir	-3.35	1	GLU 228	ARG152, ASP151, TRP179, SER180, TYR402, GLU228, ARG225, GLU277	9
11	Wogonin	-4.12	1	ASP151	ARG156, LEU 134, GLU119, TRP179, ARG118 SER180, ARG225,THR226, GLU277, GLU278,GLU228, GLU119,LEU134, TYR402	15



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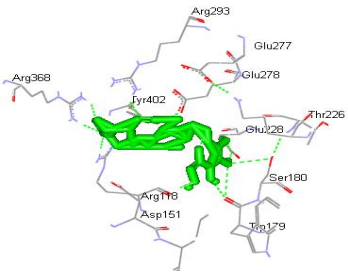

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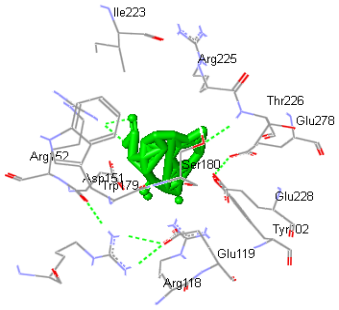

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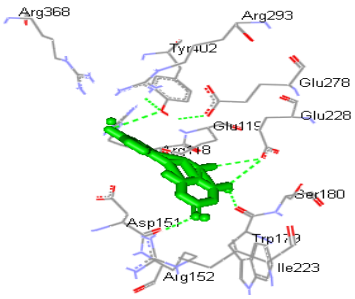

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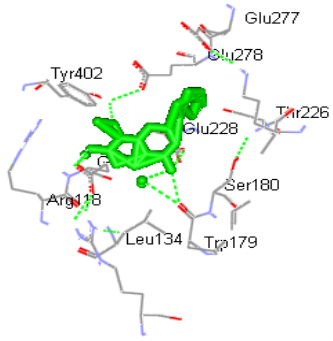

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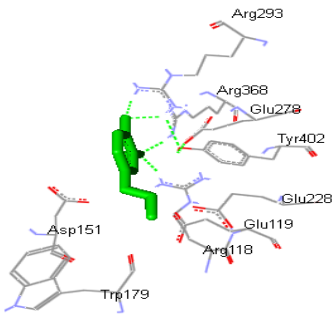

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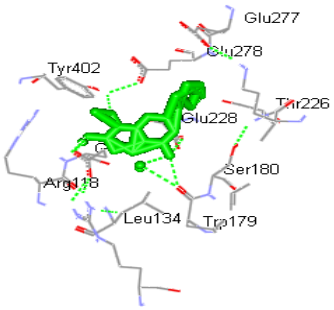

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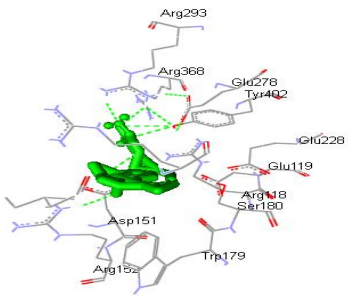
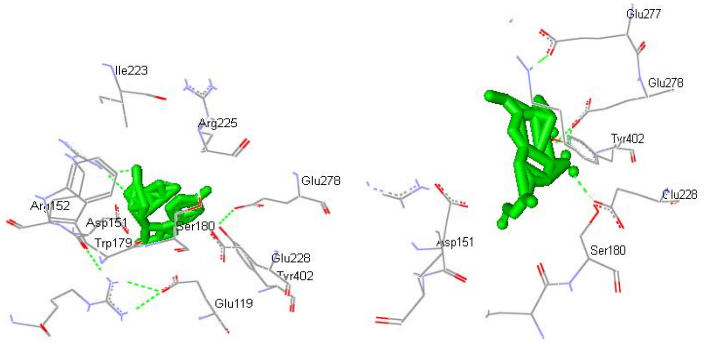


H



I J

Figure 7- 3D interactions of modeled NA protein with A) Andrograpanin, B) Neoandrographolide, C)Andrographolide, D) 14, deoxyandrographolide, E) Apigenin, F) wogonin, G) eugenol, H) Andropanoside, I) Andrographin, J) Ostelmivir

CONCLUSION

Medicinal plants have been traditionally used for treating viral infections due to their anti-viral properties. *In silico* studies of plant compounds helps in understanding the molecular mechanism of inhibition of virulence targets of viruses. In this present investigation, the major compounds of the plant *Andrographis paniculata* which have been traditionally used for swine flu infection (H1N1) were studied for their mode of interaction with the virulence target of swine flu virus. NA, the antigenic determinants of H1N1 virus was subjected to docking studies against the compounds of the plant *Andrographis paniculata.* All the natural compounds showed good binding interactions with NA protein at the active site region. Of all the natural inhibitors, andrograpanin have shown to bind with good binding energy of -8.61 kcal/mol, which could be used for invitro and invivo studies to develop as drug for treating swine flu.

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