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IN SILICO STUDY OF PHYLLANTHUS AMARUS AND OSELTAMIVIR AGAINST NEURAMINIDASE

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ABSTRACT

Objective: The aim of this study is to demonstrate the antiviral activity of *Phyllanthus Amarus* is more efficient than the commercially available drug through In Silico docking analysis. **Methods:** In this study, the structure of Neuraminidase target protein, ligand molecule of *P Amarus* phytochemical structure and anti viral drug of Oseltamivir were collected with the help of Pubchem and PDB database. The protein – ligand based molecular docking was performed with Patchdock software and visualization of macromolecules useing PyMol. **Results:** The outcome of this study speculated that the lignin group of *P Amarus* (*Phyllanthin, Phyltetralin, Niranthin, Isolintetralin, Hypophyllanthin, and Nirtetralin*) possesses greater docking scores contrasted with *Oseltamivir*. **Conclusion:** This report reveals that the lignin class of *PAmarus* Phytochemicals can be used as ligand in the preparation of antiviral drug instead of chemical based drug preparation. This needs further confirmation by invivo and invitro studies to discover the potential drug against Influenza viral infection.

KEYWORD: Antiviral, Ant influenza, *Neuraminidase,* Molecular docking, *Phyllanthus Amarus.*

INTRODUCTION

The influenza is an epizootic illness that causes a severe respiratory inflammation / disease. It's generally characterized by sudden onset of fever, muscle ache, sore throat, headache, congestion and cough. Hence, it's a very important infection because of its simplicity of

communicability, short hatching time, rapidity of viral mutation, associated ghastliness, resultant loss of efficiency, and the chance of extreme entanglements. It can be tedious, mostly in the kids, old people and in immunocompromised patients.[1]

Ethnopharmacology furnishes researchers with an elective methodology for the disclosure of antiviral specialists, in particular the investigation of restorative plants with history of conventional use as a Potential resource of substances with huge pharmacological and natural activities.[2]

WHO estimated that 80 percent of people across the world depended on herbal medicines for primary care. Medicinal plants have been found and utilized in conventional medication practice since ancient Period. It is more effective than allopathic medicines as they have fewer side effects, self-healing and are naturally available. Apart from curing diseases, the major role of plant based medicine is to improve overall health as they strengthen the whole body.[3]

Medicinal Plants combine many synthetic mixtures for capacities including protection against insects, parasites, illnesses, and herbivorous vertebrates. Various phytochemicals have been recognized with traditional biological activity or therapeutic potential. Among the vast variety of medicinal plants, *P Amarus* is one of the important medicinal plants. It's also known as Gale of the wind, is a leafy herbal plant belonging to the Kingdom - *Plantae*, Order – *Malpighiales*, Family - *Phyllanthaceae*, Genes – *Phyllanthus*. It is the native plant of tropical regions in the Americas, Africa, India, China and SouthEast Asia.[4] Recent studies on P Amarus have given the assurance that it is rich with phytochemicals such as Flavonoids, Lignin, Triterpenes, Tannins and Alkaloids.[5]

All parts of this plant (seed, leaves, flower and root) have been reported with many pharmacological actions such as Antimicrobial, Antioxidant activity, Anti diabetic, Anticancer, Anti-inflammatory and Hepatoprotective effect. It also cures gallstones and kidney stones, even bronchitis and anaemia.[6]

Docking is a computational procedure of binding orientation of compounds and their biological targets like ligand and protein which results in the formation of a stable complex compound.[7] It is an easy, quick, accurate and cost-effective process used by many researchers in various research fields. Molecular Docking enables us to assess the binding

ability of ligands to protein active sites.[8]

The objective of the present study is to validate Antiviral activity of *P Amarus* phytochemical component with *Neuraminidase* of *Influenza* Virus and its docking score was compared with Antiviral drug *Oseltamivir*.

MATERIALS AND METHODS

In this, In Silico study phytochemical components of *Phyllanthus Amarus*[8] structures were identified from the Pubchem database [https://pubchem.ncbi.nlm.nih.gov/]. Preparing the ligand involves ensuring that its atoms are assigned the correct order. This is the crucial step before proceeding to Docking. The molecule should be with Zero violation and it should obey Lipinski Rule of Five [https://www.molinspiration.com] and good Pharmacokinetic activity [http://www.swissadme.ch/]. Ligand molecules of 10 phytochemical components from *P Amarus* and *Oseltamivir* were used in Cheminformatic tools for online smile translator [https://cactus.nci.nih.gov/]. This is done in order to convert the Smiles Strings to a pdb format file. To visualize the resulting ligand the Pymol software was used (Fig 1).

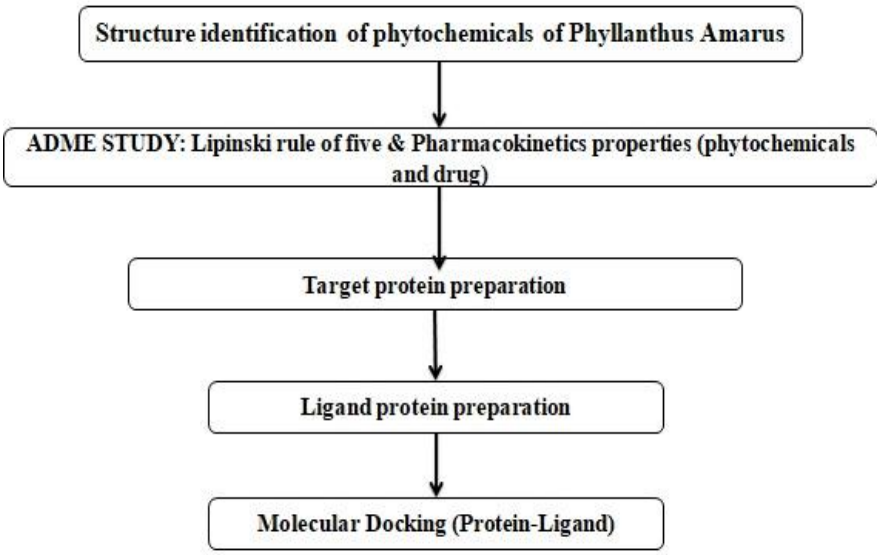


Figure 1: Workflow of the study

Neuraminidase is selected as target protein, because it is one of the important proteins in Influenza virus. The structure was identified (Template, Sequence identity, GMQE (global model quality estimate), QMEAN scores from pdb database [https://www.rcsb.org/] and downloaded the FASTA format (with the help of Swiss model database and get the pdb

format). Docking of target protein (Neuraminidase) and ligand (Phytochemicals and Oseltamivir) were programmed via Patchdock software [https://bioinfo3d.cs.tau.ac.il/PatchDock].

RESULT AND DISCUSSION

Influenza virus infection causes Flu, which attacks nose, throat and lungs in adults and children that leads to chronic medical conditions. Due to this severity, the present study was focused on the analysis of a phytochemical based Antiviral component. It was planned through In silico study of protein ligand docking through Patchdock [http://bionfo3d.cs.tau.ac.il/PatchDock].

Structural Identification of Phytochemicals of Phyllanthus Amarus

Phytochemical components structures were recovered from pubchem data set [https://pubchem.ncbi.nlm.nih.gov]. These components, pubchem ID, 2D and 3D structures were listed in Table 1.

ADME Study

ADME provides pharmacokinetic properties of a molecule. It is used to investigate metabolism of a chemical or a drug in living organisms. It is helpful in understanding the safety and efficiency of Drug molecules.[12]

Lipinski Rule of Five - Phytochemicals and Oseltamivir

Lipinski Rule of Five helps to evaluate the chemical component with a specific Pharmacological or Biological activity that has properties that are responsible for being an orally active medication.

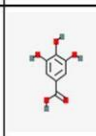
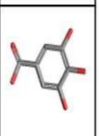
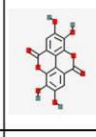
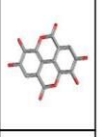
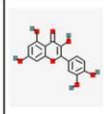
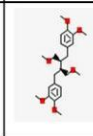
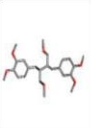
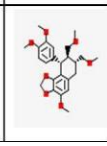
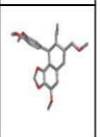
It should follow the conditions listed below

- ✓ Molecular mass should be below 500 Daltons
- ✓ Hydrogen bond acceptor should be under 10
- ✓ Hydrogen bond donor should be under 5
- ✓ Log P (Octanol water Partition coefficient) should not be more than 5

The rule depicts molecular properties important for a medication's pharmacokinetics. Among 11 selected phytochemicals of *P Amarus* except *Quercetin* (hydrogen bond donor is 5 which doesn't come under the rule) all other phytochemicals and *Oseltamivir* obeys Lipinski Rule of Five and are listed in Table 2. The properties are identified by utilizing Molinspiration

Cheminformatics [https://www.molinspiration.com] tool.

Table 1: Structures of Phytochemicals of Phyllanthus Amarus.

Sl. No	Component Name	Chemical formula	Pubchem ID	2D structure	3D Structure
1	Gallic acid	$C_7H_6O_5$ or $C_6H_2(OH)_3COOH$	370		
2	Ellagic acid	$C_{14}H_6O_8$	5281855		
3	Quercetin	$C_{15}H_{10}O_7$	5280343		
4	Phyllanthin	$C_{24}H_{34}O_6$	358901		
5	Hypo-phyllanthin	$C_{24}H_{30}O_7$	182140		

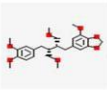
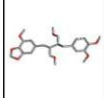
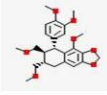
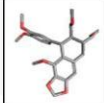
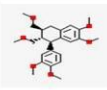
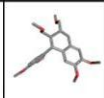
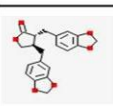
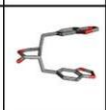
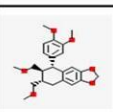
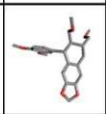
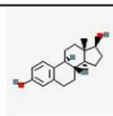
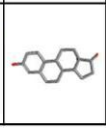
Sl. No	Compound Name	Chemical formula	Pubchem ID	2D Structure	3D Structure
6.	Niranthin	$C_{24}H_{32}O_7$	13989915		
7	Nirtetralin	$C_{24}H_{30}O_7$	182644		
8	Phyltetralin	$C_{24}H_{32}O_6$	11223782		
9	Hinokinin	$C_{20}H_{18}O_6$	442879		
10	Isolintetralin	$C_{23}H_{28}O_6$	101241675		
11	Estradiol	$C_{18}H_{24}O_2$	5757		

Table 2: Lipinski Rule of Five of *Phyllanthus Amarus* Phytochemicals and *Oseltamivir*.

Sl. No	Compound Name	Molecular weight g/mol	Hydrogen bond acceptor	Hydrogen bond donor	Mi log P
1	Gallic acid	170.12	5	4	0.59
2	Ellagic acid	302.19	8	4	0.94
3	Phyllanthin	418.53	6	0	3.92
4	Hypophyllanthin	430.50	7	0	3.50
5	Niranthin	432.51	7	0	3.95
6	Nirtetralin	430.59	7	0	3.70
7	Phyltetralin	416.51	6	0	3.47
8	Hinokinin	354.56	6	0	3.02
9	Isolintetralin	400.47	6	0	3.72
10	Estradiol	272.39	2	2	3.42
11	Oseltamivir	312.41	6	3	0.85

Pharmacokinetic properties of phytochemicals and *Oseltamivir*

Pharmacokinetic study explains the rate of extent at which a drug reaches the site of action within an organism. Randy R. Milleret et al[13] express that the body affected a specific chemical or a drug after administration through the mechanism of adsorption and distribution.[13]

By applying Pharmacokinetic Principles to preclinical trials, safer and exact clinical trials can be planned. The pharmacokinetic properties of phytochemicals and drug were calculated by utilizing the SwissADME database [http://www.swissadme.ch/] and it's listed in Table 3.

Table 3: Pharmacokinetic properties of Phytochemicals of *Phyllanthus Amarus* and *Oseltamivir*.

Sl.No	Component	ESOL log S	ESOL Class S	GI Absorption	BBB Permeability	Bioavailability score
1	Gallic acid	-1.64	Very soluble	High	No	0.56
2	Ellagic acid	-2.94	Soluble	High	No	0.55
3	Phyllanthin	-4.53	Moderately soluble	High	Yes	0.55
4	Hypophyllanthin	-4.55	Moderately soluble	High	Yes	0.55
5	Niranthin	-4.59	Moderately soluble	High	Yes	0.55
6	Nirtetralin	-4.55	Moderately soluble	High	Yes	0.55
7	Phyltetralin	-4.54	Moderately soluble	High	Yes	0.55
8	Hinokinin	-4.41	Moderately soluble	High	Yes	0.55
9	Isolintetralin	-4.47	Moderately soluble	High	Yes	0.55
10	Estradiol	-4.28	Moderately soluble	High	Yes	0.55
11	Oseltamivir	-1.88	Very soluble	High	No	0.55

The ESOL refers to the Estimation of Aqueous Solubility of the component; the moderate value of ESOL of a ligand should not exceed 6 and should be soluble.[14] All the selected phytochemicals and drug are noted to be less than -5 and *Gallic acid, Ellagic acid* and the dug *Oseltamivir* are very soluble, all other Phytochemicals are moderately soluble.

From this result, it is recorded that *Gallic acid, Ellagic acid and Osteltamivir* were not crossing the Blood Brain Barrier. The BBB permeability protects the brain by avoiding the circulation of toxins or pathogens that causes brain infection.[16]

This result showed that the Gastro Intestinal (GI) absorption of all the phytochemical components was recorded to be high. This parameter is important to understand the transportation and absorption rate of Drug molecules.[15]

From this report, the Bioavailability score (the rate of extent of drug absorption) of all the Phytochemicals and Drug were noted to be 0.55 (except *Gallic acid* - 0.56). A neutral organic compound to act as a good oral drug their Bioavailability score should be 0.55[15] Then it can be successfully used as a ligand for docking studies.

Target protein preparation

In this present study, we selected the target protein as *Neuraminidase* from *Influenza* virus. Because, it is responsible for splitting the receptor of the influenza virus that allows the replication of virus in the host cell. The FASTA sequence of *Neuraminidase* was retrieved from the PDB database (ID-4WA3) [https://www.rcsb.org/]. Through the Swiss Model database the pdb format of the Target Protein was developed [http://www.swissadme.ch/].

Z- Score

Modelling of Protein structure was done by Swiss Model database as the quality assessment of Protein structure plays a crucial role in structure validation and prediction.[17] The structure of *neuraminidase* is shown in **Fig2a** and QMEAN Z-score is listed in **Fig 2b.**

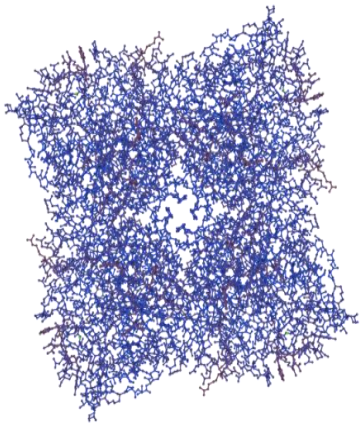


Figure 2a: Structure of Neuraminidase

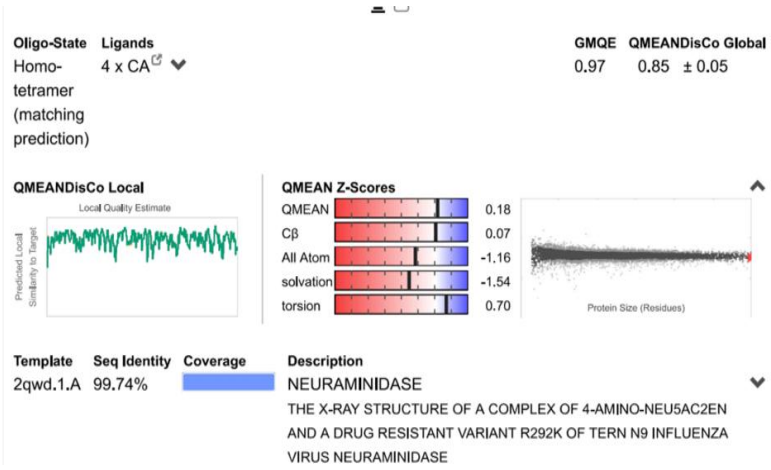


Figure 2b: QMEAN Z- Score value

Z-scores about 0.0 - therefore contemplate a Native-like structure, a QMEAN Z-score under -4.0 indicates a model with less quality and higher QMEAN Z score value means better agreement with the predicted feature.[17] From the result, GMQE value was 0.97, QMEAN value was 0.18, solvation value was -1.54, and torsion value was 0.70 (**Fig: 2b**).

Ligand preparation

As per the Lipinski Rule of Five and based on violation (zero violation) we select the 10 phytochemicals component and Osteltamivir for ligand preparation (Table 2). These

structures Smile sequence were collected from ncbi Pubchem and the pdb file was retrieved with the help of online SMILES translator tool Cactus [https://cactus.nci.nih.gov/]

Molecular Docking (Protein-Ligand)

Docking is a tool in computational drug design to know a particular compound as antagonist for a particular protein. The compounds with the good docking results will be integrated and investigated in the laboratory.[18]

From this Docking work, the selected phytochemicals (Phyllanthin, Hypophyllanthin, Niranthin, Nirtetralin, Phyltetralin, Isolintetralin, Gallic acid, Ellagic acid, Hinokinin, Estradiol) and the Osteltamivir as ligand were docked against Target protein Neuraminidase by utilizing the PatchDock tool [http://bionfo3d.cs.tau.ac.il/PatchDock]. The structure and active site of Target protein where the ligand binds was retrieved from Pymol software. The scores obtained from Docking (PatchDock) of Oseltamivir and phytochemicals of *Phyllanthus Amarus* against *Neuraminidase* are illustrated in **Fig 3 and 4.**

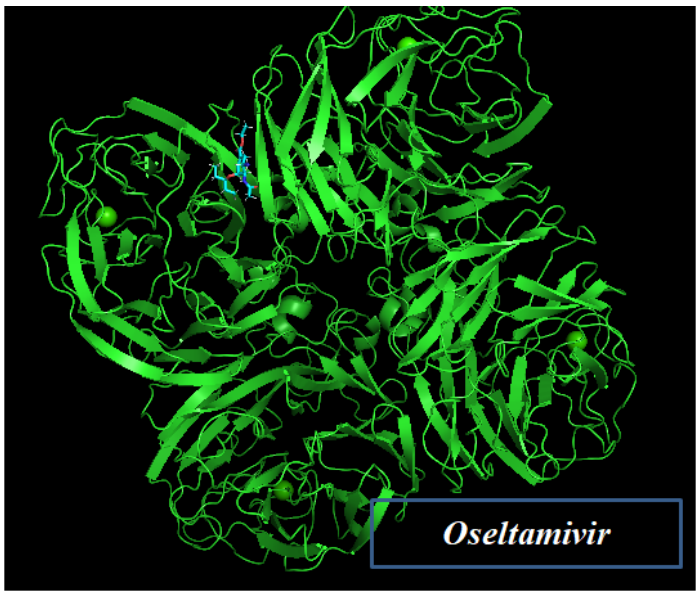


Figure 3 - Molecular Docking analysis of Neuraminidase with *Oseltamivir*.

Some of the previous studies showed antiviral effects in phytochemical compounds. Z Zakay-rones et al.,[19] showed that *elderberry* syrup was effective against *Influenza A* virus illness.[19] One of the recent antiviral docking study disclose that the DNY peptide ligand has the negative ΔG b interactive value and it has a powerful bond on the *Neuraminidase* contrast to the other two peptide enzymes (NNY and LRL).[20]

T P Krishna Murthy et al, and Shridhar Hiremat et al,[8;21] proclaimed that the phytochemicals of *P Amarus* were also effective against SARS-CoV-2 main protease. Vincent S etal.,[22] hypothesize that the compounds of Kabasura Kudineer (KK) make use of its antiviral properties against novel coronavirus SARS CoV-2 by either blocking the host cell receptor or hold back the key viral protease required for its reproduction in the host cell.[22]

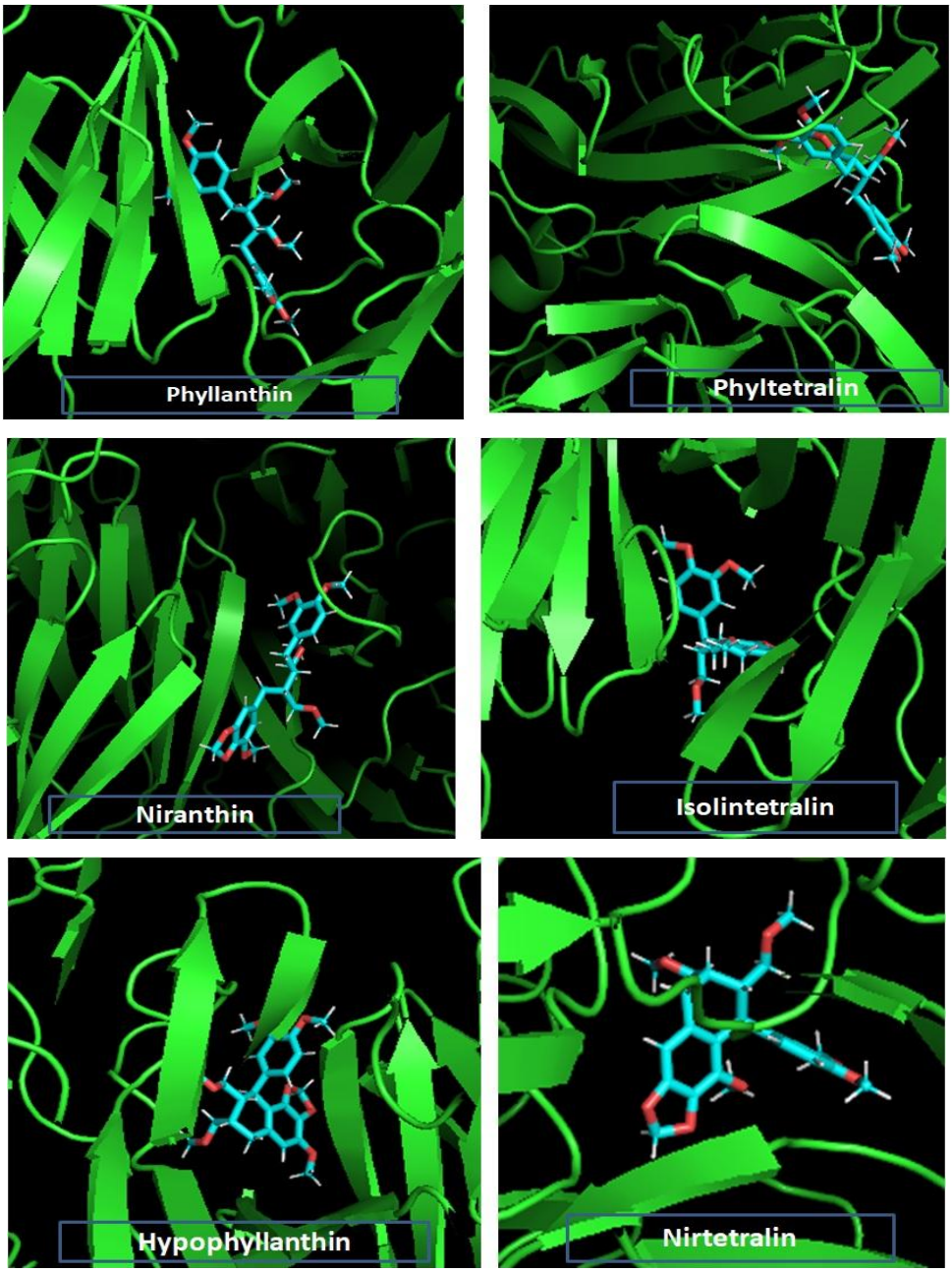


Figure 4: Molecular Docking analysis of Neuraminidase with Phyllanthin, Phyltetralin, Niranthin, Isolintetralin, Hypophyllanthin, and Nirtetralin.

Mekha Mohan et al[23] showed that bioactive compounds of total anthocyanin, phenolic and flavonoids have high activity against Hepatitis B DNA polymerase and effective on

nucleocapsid protein of white spot syndrome virus.[23]

CONCLUSION

From the past several years through various studies phytochemicals urge to be more efficacious drug used for the treatment of many diseases. This Insilco study on *P Amarus* ventured to look over the potency of phytochemicals as a ligand in the inhibition of target protein Neuraminidase of Influenza virus. From this docking study, it is speculated that the phytochemicals were more effective against the *Neuraminidase* (Target protein). Especially, phytochemicals belonging to Lignin group (Phyllanthin-5650, Hypophyllanthin-5256, Niranthin-5342, Nirtetralin-5060, Phyltetralin-5650, Isolintetralin-5328) have high docking score compare with the currently practicing antiviral drug *Oseltamivir*-4678. It shows these phytochemicals are more effective than the drug. The commencements of natural antiviral agents that are environmentally friendly and non-pollutant or contaminate the environment are to be anticipated.

Hence we conclude that, in future it should be focused on the preparation of drugs through phytochemicals instead of man-made chemical components as they are reported to be less toxic and have fewer side effects comparatively. This can be investigated more through in vivo and preclinical testing for further endorsement.

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