In Silico Investigation of Indian Medicinal Herbs for Influenza Management: A Focus on *Ocimum sanctum*, *Withania somnifera*, and H1N1 Neuraminidase Targeting

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Pinki

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Under the Supervision of

Dr. Navneeta Bharadvaja

Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042,

June, 2025

DELHI TECHNOLOGICAL UNIVERSITY

(formely Delhi College of Engineering)

Shahbad, Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I, Pinki, 23/MSCBIO/37 say that the work shown in this thesis entitled **'In Silico Investigation of Indian Medicinal Herbs for Influenza Management: A Focus on *Ocimum sanctum*, *Withania omnifera*, and H1N1 Neuraminidase Targeting'**, that submitted for the Master of Science degree at Delhi Technological University, is a genuine account of my own work. I did the work with the help of the supervision of Dr. Navneeta Bharadvaja during the period from January 2025 to June 2025.

I affirm that I did the work alone and I have not used the work or the words of others in any part of this paper for an award of any kind .

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DELHI TECHNOLOGICAL UNIVERSITY

(formely Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-42

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Certified that **Pinki (23/MSCBIO/37)** has successfully accomplished search work presented in this thesis, entitled **'In Silico Investigation of Indian Medicinal Herbs for Influenza Management: A Focus on *Ocimum sanctum*, *Withania somnifera*, and H1N1 Neuraminidase Targeting'** for the award of **Master of Science** from Department of Biotechnology, Delhi Technological University, under my supervision. The findings and results included in this thesis are derived from the student's independent and original research and the content has not been used previously either by the candidate or any other person, for the conferment of a degree or diploma at Delhi Technological University or any other academic institution.

Prof. Yasha Hasija
Head of Department
Department of Biotechnology
Delhi Technological University

Dr. Navneeta Bharadvaja
Supervisor
Department of Biotechnology
Delhi Technological University

Date: 5/6/2025

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Pinki

ABSTRACT

Influenza, classified within the Orthomyxoviridae family cause significant illness and death worldwide.The H1N1 subtype of influenza, causative of 2009 swine flu pandemic, triggered respiratorty tract infections in multiple species, including human, birds and swine is highly adaptable virus. It has a segmented genome , consisting of eight RNA segments. It undergoes rapid evolution through gradual accumulation of minor genetic changes in viral RNA (antigenic drift) and major genomic rearrangement through ressortment of RNA segments (antigenic shift), which leads to emergence of new strain enabling the H1N1 to dodge immune response from the host and reduce impact of antiviral drugs. There are two critical surface protein hemaglutinin (HA) which mediates entry by recognizing sialic acid receptors present on host cells and neuraminidase(NA) ,which cleaves sialic acid residues to release the viral progeny .Currently NA targeting medications such as Oseltamivir(marketed as Tamiflu) and Zanmivir serve as effective .But due to its low efficacy in resistant H1N1 strains, has created an urgent need for novel antiviral compound with improved efficacy and resistance profile.

This study aims to identify potential drug candidate derived from *Withania somnifera and Ocimum sanctum* ,targeting Neuraminidase using in silico approach called molecular docking. According to the docking studies, among all tested compounds, Withaferin A (Pubchem ID : 265237) demonstrated the most favorable overall pharmacological profile. Compared to Oseltamivir (Pubchem ID : 65028) with binding affinity -6.5 kcal/mol with NA and other phytocompounds, Withaferin A reflected the most negative binding affinity i.e. −9.2 kcal/mol, indicating a highly stable interaction with the NA. In ADMET profiling Withaferin A exhibited no Lipinski rule violations, high gastrointestinal (GI) absorption, acceptable bioavailability (0.55), and minimal CYP enzyme inhibition. Moreover, it is non-mutagenic (Ames negative) and non-hepatotoxic, which are critical safety parameters. Being a phytochemical further adds to its drug-likeness potential. In contrast, Oseltamivir which is a synthetic drug and other phytocompounds either showed lower binding energy, Lipinski violations, poor GI absorption, or undesirable toxicity profiles. Thus, Withaferin A emerges as the most promising lead compound for further development.

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LIST OF ABBREVIATIONS

1. HA: Hemagglutinin
2. NA: Neuraminidase
3. ADMET: Absorption, Distribution, Metabolism, Excretion ,Toxicity
4. pkCSM:Pharmacokinetics and Cheminformatics for Small Molecules.
5. PA:called Polymerase Acid Protein
6. PB1: Polymerase Basic Protein type 1
7. PB2: Polymerase Basic Protein type 2
8. M1: Matrix Protein type 1
9. M2: Matrix Protein type 2
10. NS1: Non Structutal Protein type 1
11. NS2: Non Structural Protein type 2
12. NP: Nucleoprotein
13. RNP: Ribonucleoptotein
14. RNA: Ribonucleic Acid
15. EGCG: Epigallocatechin gallate
16. HIV: called Human Immunodeficiency Virus
17. SARS-CoV: called Severe Acute Respiratory Syndrome Coronavirus
18. HSV: Herpes simplex virus
19. HCV: Hepatitis C-Virus
20. RSV: Respiratory Syncytial-Virus
21. EGCG: Epigallocatechin gallate
22. BBB: Blood Brain Barrier
23. PDB: Protein Data Bank
24. CYP: Cytochrome P450
25. GI: Gastrointestinal
26. ROS: Reactive Oxygen Species
27. TPSA: Topological Polar Surface
28. Mol. Wt. : Molecular Weight

CHAPTER-1

INTRODUCTION

1.1 BACKGROUND

Influenza Virus contribute to common seasonal illness as well as large-scale pandemics.The influenza outbreak of 1918, led to the deaths of over 40 million people worldwide and swine flu pandemic of 2009, again swept the across the globe, causing upwards of over 200 thousands. They have capability to possess acute respiratory infections in both humans and animals. Prompt mutation and genetic reassortment which leads to novel strains with different antigenicity and pathogenic potential keeps the virus a public health concern (Taubenberger & Morens, 2008). The virus relies on two critical glycoproteins for infection: Hemagglutinin & Neuraminidase . While HA is central for cell entry, NA cleaves sialic acid residues on host cells and viral envelopes allowing for new budding viruses to infiltrate other cells(Gamblin, S. J., & Skehel, J. J. ,2010) For the treatment and management of Influenza infections two antiviral agents are most effective, oseltamivir (Tamiflu) and zanamivir, both NA inhibitors. The function of these drugs is to bind to the active site of NA, thus inhibiting virulent replication and dissemination (Moscona, 2005) . But, the overuse of these antivirals has led to various H1N1 strains Oseltamivir-resistant, which lowers the overall effectiveness of the drug.

The most common point mutation of the NA gene, the H275Y mutation, is known to cause resistance by changing the binding of the drug without completely destroying the function of the enzyme (Hurt et al., 2009; Bloom et al., 2010). This has created an urgent need for more efficient antiviral drugs which contend with resistance, have greater effectiveness, and can assist in controlling the advancements of viruses. A particular class of secondary metabolites termed phytocompounds are gaining prominence as interest in plant-based medicines continue to expand further. Numerous bio-active compounds including flavonoids, alkaloids, polyphenols, and terpenoids have showed antiviral activity against several strains of viruses (Jassim & Naji, 2003; Lin et al., 2014). Their mode of actions includes the inhibition of virus essential enzymes, suppression of immune response regulations, and blockade of viral internalization and viral genomic replication. Due to vast array of chemical structures that these phytocompounds possess, there exists a strong potential of developing new antiviral drugs. *Withania somnifera* & *Ocimum sanctum* are Indian medicinal plants having a wide variety of phytocompounds exhiting therapeutic effects. The rise of computational biology and bioinformatics has transformed the preliminary step of drug development. This created an opportunity for researchers to concentrate on the screening and characterizing natural phytocompounds that could potentially target and inhibit Influenza A (H1N1) neuraminidase, making them more effective and safer than oseltamivir.

Molecular docking techniques enable the rapid and economical and high throughput profiling of compound libraries to assess their potential binding affinity with specific biological targets of interest. During the preliminary ADMET evaluation, researchers perform an initial

screening to identify candidates with favorable pharmacokinetics and minimal toxicity prior to animal model and cell culture based study. This research attempts to analyze if some phytochemicals could be useful in combating H1N1 by merging traditional approaches with modern scientific frameworks. This study aims to find stronger candidates for antiviral pharmaceuticals by comparing the docking efficiency and ADMET characteristics of the screened compounds to those of oseltamivir.

1.2 OBJECTIVE OF THE STUDY

The study seeks to rationally design candidates more effective and safer than oseltamivir for Influenza A (H1N1) neuraminidase utilizing natural phytocompounds from *Withania somnifera and Ocimum sanctum* by screening and evaluating antiviral phytocompounds. The main steps are:

1. Search and evaluate the available phytocompound with antiviral activity.
2. Employ in silco, structure based molecular docking to determine potential binding propensity between target and ligand.
3. Compare the result of the docking studies with oseltamivir, which is the reference drug.
4. Perform ADMET analysis of the top phytocompounds to study the pharmacokinetics, drug-likeness, and toxicity potential.

To select the promising top-most drug-like Candidates for future in vitro and in vivo screening and development into anti-influenza drug candidates

CHAPTER-2

LITERATURE REVIEW

2.1 INTRODUCTION

influenza Viruses, classified within the family known as Orthomyxoviridae, are envelope containing ssRNA Viruses. These viruses are significant pathogens, responsible for infecting a varied array of species including human and animals. These Viruses are classify in 4 major Types: A, B, C and D. Type A influenza and Type B cause recurrent seasonal flu outbreaks. While type known for its notorious role in triggering the most severe epidemics and pandemics like swine flu pandemic of 2009. Influenza A Type Viruse further subtyped on the basis of their surface protein HA and NA such as H1N1 and H3N2. Type B is categorizes into lineages such as B/Yamagata and B/Victoria (Dangi & Jain,2012). It is known for its complex viral morphology that helps in host invasion and viral proliferation. In this thesis we focuses on H1N1 influenza virus, having highly complex structure which enables efficient infection and viral proliferation.

2.2 STRUCTURE OF H1N1

The H1N1 with ssRNA with negative polarity consists of 8 segments. These segments code for one or more key viral proteins Name as PA,HA, PB1, PB2 , NP, NA, M1/M2 and NS1/NS2.. These segments are encapsulated in RNP complex, NP and viral polymerase components. (Dadonaite ,2019) M1 protein found underneath the lipid envelope and helps in viral assembly and provide structural stability. The M2 ion channel protein helps in pH dependent uncoating of the virus inside endosomes(Chlanda et al,2015) , NS1 suppresses host's immune response and NS2 (NEP) function in nuclear export of ribonuceoproteins(RNP) (Salahuddin & khan,2020).

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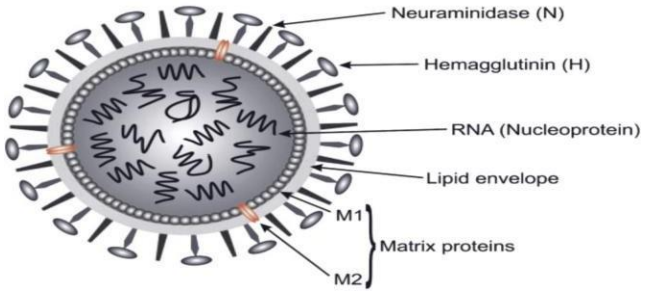

Figure 1 Structure of H1N1

2.3 REPLICATION MECHANISM

The replication mechanism of H1N1, like other Influenza viruses, is multi step and occurs within the host cell(Cytoplasm & Nucleus).

2.3.1 Attachment and entry

H1N1 attaches to the cell surface sialic acid receptors found on the respiratory epithelial cells via HA protein. The attachment triggers receptor- mediated endocytosis, allowing the entry of the virus inside an endosome.

2.3.2. Uncoating

A drop in pH triggers changes in HA conformation and it activates the M2 Type ion channel. that facilitate the entry of proton (H+) inside the virion, resulting in uncoating . The Viral ribonucleoprotein released into cell cytoplasm and then transported inside the nucleus.

2.3.3. Transcription and Replication (in the nucleus)

Influenza replicates in the nucleus of the host cell. RNA- dependent RNA polymerase (composed of polymerase basic type 1,polymerase basic type 2 and polymerase acidic (PA) proteins transcribes -ssRNA into +mRNA. PB2 attaches to capped host pre mRNAs and steals the cap (cap snatching), allowing viral mRNA to be detected by host ribosomes.

The polymerase replicates full length cRNA, which serves as a template to make more vRNA copies. These vRNA are packaged into virion in later stages.

2.3.4. Translation

Viral mRNA reaches to cytoplasm from the nucleus, where host ribosomes translated them. Viral proteins like hemagglutinin, neuraminidase, and M2 are translated and released to the endoplasmic reticulun and then ti the PM. While internal proteins such as nucleoprotein, polymerase basic 1, polymerase basic 2, PA, M1, NS1all are transported back to the nucleus or used in packaging of new virion particles.

2.3.5. Assembly

Newly synthesized vRNPs are assembled in the nucleus and transported into the cell by the facilitation of the M1 and NEP (NS2) proteins. They move to cell membrane, where the viral proteins already presented.

2.3.6. Budding and Release.

Newly formed enveloped virus buds off from the Host cellular membrane. Neuraminidase Break residues of Sialic acid (SA) on the host cell surface to prevent clumping and release the new virions.

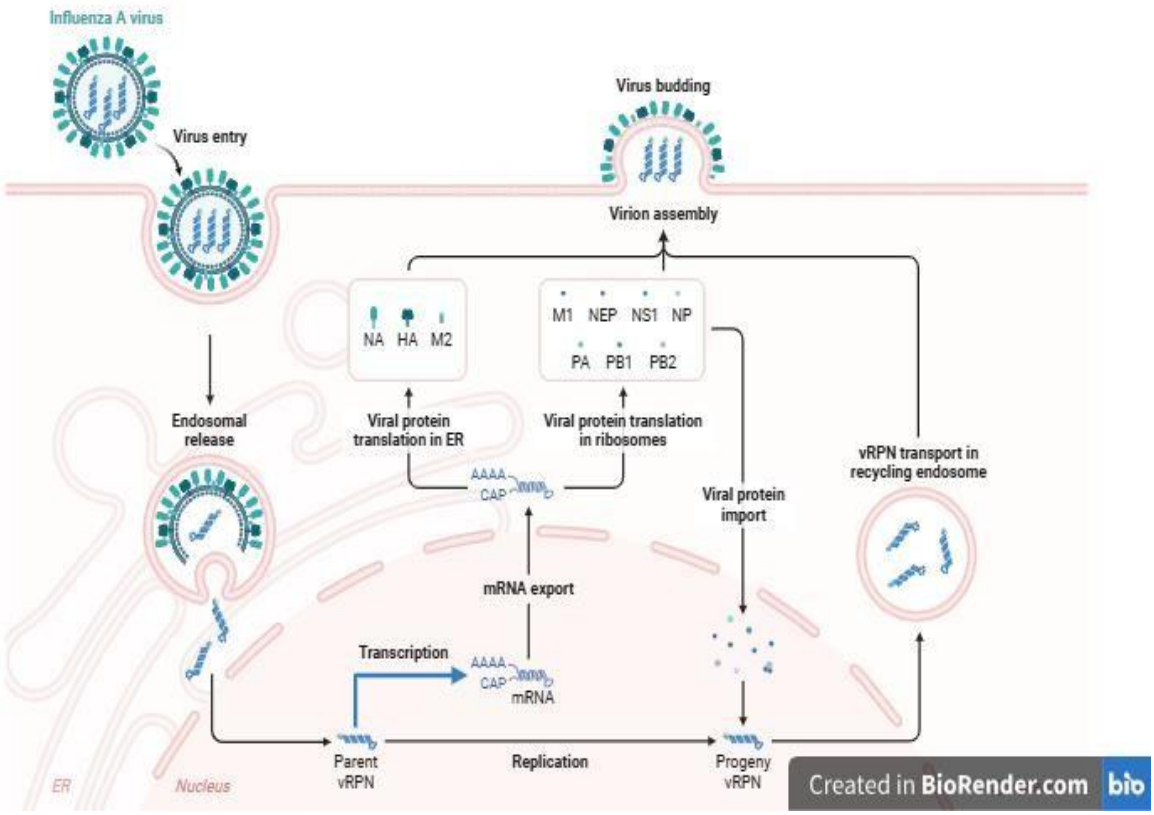

Figure 2 : Mechanism of Replication of H1N1 influenza

2.4 Mode of transmission

The H1N1 virus disseminated through aerosolized respiratory secretions from upper respiratory tract emitted during verbal communication, sneezing or coughing episodes. It can also be spread through fomites and indirectly by contact through hand-to-mouth or hand-to- nose interactions (Belser et al., 2009). Transmission through aerosols has been shown to occur in confined spaces which are poorly ventilated and during certain medical procedures that generate an aerosol (Neumann & Kawaoka, 2015).

2.4.1 Cross-Species Transmission:

H1N1 exhibits cross-species infectivity in a range of multiple host species, notably humans, swine and avian host. Its pandemic strain in 2009 stemmed from a fusion of avian, swine, and human viruses, showcasing the importance of cross-species infection in the spread of a pandemic (Donatelli et al., 2011; Torremorell et al., 2014). The success of interspecies transmission depends on receptor binding; H1N1 has a preference for α2,6-linked sialic acid receptors dominantly present in the human upper respiratory tract (Hui et al., 2010).

2.4.2 Animal Models and Experimental Evidence:

Studying the H1N1 virus requires animal models like ferrets, guinea pigs, and macaques. These models replicate the human infection and demonstrate how temperature, humidity, and specific alterations to hemagglutinin (HA) and neuraminidase (NA) could influence transmission (Bouvier & Lowen,2010; Thangavel & Bouvier,2014)

2.4.3.. Anthropozoonotic and Public health consequences:

H1N1 is endemic in pigs and there is a chance that new viral strain will reassort and cause infection in humans. This shows the emergence of surveillance and integrates "One Health" approaches for early detection and pandemic preparedness.(Short et al., 2012; Webstee et al., 2007).

In essence, H1N1 transmission is influenced by adaptability of virus, host susceptibility, and environmental conditions, with significant implications for pandemic preparedness and zoonotic spillover.

2.5 OSELTAMIVIR AS NEURAMINIDASE INHIBITOR

Oseltamivir is an antiviral prodrug, administered orally. It is converted into oseltamivir carboxylate (pharmacologically active form) during first-pass hepatic metabolism. Active oseltamivir (oseltamivir carboxylate) acts as a selective inhibitor of neuraaminidase enzyme in H1N1. When it reaches in systemic circulation, mimics natural sialic acid substrate and inhibits NA from cleaving sialic acid residues. It reduces viral load and symptom severity via preventing the release of new viral progeny(He et al.,1999). Oseltamivir is fastly absorbed, and shows significant systemic levels of the active compound within 2-3 hours post administration. It is eliminated via the kidneys predominantly, having an elimination half-life of 6-10 hours approximately (Wattanagoon et al., 2009; Davies, 2010). Oseltamivir is effective in both prophylactic and therapeutic context but mutations which alter the AA sequence in the enzyme's binding site of neuraminidase gene create resistance to Oseltamivir. The most common mutation is H275(His to Tyr at position 275), in which NA enzyme function is retained but reduces the oseltamivir's binding affinity . It was reported during 2008-2009 flu season and associated with community transmission(Govokova et al.,2010).When I223R(isoleucine to Arginine at position 223) mutation combines with H275Y reduces susceptibility to oseltamivir and other neuraminidase inhibitors, enhances the replication of virus which leads to increased virulence(Song et al.,2011). These mutation cause challenges for the management of influenza in immunocompromised patients and settings with overuse of oseltamivir.

2.6 INDIAN MEDICINAL PLANT AS THERAPEUTIC AGENT

Indian Medicinal plant flora have long served as a potent source of bioactive compounds exhibiting therapeutic effects. Traditional Ayurvedic practices have used these plants to treat viral infections and are increasingly supported by contemporary scientific research, which validate the antiviral effectiveness of numerous phytocompounds. Some species like Ocimum sanctum(Tulsi), Tinospora cordifolia(Giloy), Andrographis paniculata(kalmegh) , Withania somnifera(Ashwagandha) contain naturally occurring secondary metabolite like terpenoids, flavonoids, alkaloids, and polyphenols, which exhibited inhibitory effect against viruses such as hepatitis, herpes, humanpapilomavirus(HPV) by supressing the viral entry, replication and protein synthesis. Integraring old traditions with high-throughput screening for drug development and molecular docking techniques shows that these plant could be used as strong candidate for the development of powerful antiviral drugs. (Saxena, 2021).

Phytocompounds from medicinal plants are secondary metabolite has been used for wide range therapeutic application. These compounds include flavonoids, alkaloids, phenolic acids, saponins and terpenoids. Each has different effects. For example quercetin and kaempferol under flavonoids have antioxidant, anti-inflammatory and antiviral properties. Berberine, an alkaloids shows significant antidiabetic and antimicrobial activities. Curcumin and andrographolide have anticancer, anti-inflammatory and antiviral properties via modulation of intracellular signalling. Saponnins can boost the immune system and protect against viruses. On the other hand tannins and phenolic compounds reduce oxidative stress and supports the antioxidant defenses and protect the Liver. These bioactive compounds act through various mechanisms like inhibiting virus from replication, scavenging of ROS, modulation of immune system and regulation of gene expression. While their potential is well supported, clinical translations faces challenges like standardization, bioefficacy and regulatory approval (Patel et al., 2020). Plant derived phytocompounds known to have antiviral effects. Flavonoids(Quercetin, Luteolin, Apigenin, Kaempferol) , Alkaloids(Berberine, Lycorine, Sanguinarine) and Terpenoids(Glycyrrhizin, Andrographolide, Artemisinin), Polyphenols(EGCG) known to have antiviral effect. Here is a detailed overview showing the antiviral effect of phytocomponds, obtained from their plant sources.

Phytocompound	**Plant source**	**Target viruses**	**Mechanism of action**	**Reference**
Quercetin	Onion,apples, capers	Influenza A, Dengue, SARS-CoV-2	Inhibits viral polymerases; blocks viral entry	(Colunga,Biancatelli, 2020)
Luteolin	Celery,thyme	Japenes encephalitis virus, HIV	Inhibits viral replication	(Choi,2012)
Apigenin	Parsley,Chamomile	HSV, HCV	Inhibits viral gene expression	(Zhang, 2017)
Berberine	Berberis species	HSV, Influenza , HCV	Inhibits DNA/RNA synthesis	(Yu et al.,2005)
Lycorine	Amaryllidaceae plant	SARS-CoV, poliovirus	Inhibits viral replication	(Li et al.,2005)
Glycyrrhizin	Licorice root	SARS-CoV, Hepatitis viruses	Inhibits viral entry and replication	(Cinatl et al, 2003)
Betulinic acid	Birth bark	HIV, Influenza	Disrupts viral maturation	(Pisha et al.,1995)

Andrographolide	*Andrographis paniculata*	Dengue virus, Influenza	Modulates host immune response, blocks replication	(Panraksa et al., 2013)
EGCG	Green tea(*Camellia sinensis)*	HIV, Influenza, SARS-CoV-2	Binds to viral envelope proteins, inhibits fusion	(Steinmann et al., 2017)
Resveratrol	Grapes, peanuts	RSV, HSV, MERS-CoV	Suppresses viral protein synthesis.	(lin et al., 2017); (Martinez et al.,2020)
Saikosaponins	Bupleurum species	Influenza, coronavirus	Inhibits viral entry	(Cheng et al.,2006)
Ginsenosides	Ginseng (Panax species)	RSV, Influenza	Modulate immunity, block replication	(Kang et al.,2012)
Curcumin	Turmeric	SARS-CoV-2, Influenza, HSV	Inhibits protease, anti-inflammatory	(Praditya et al.,2019)
Withaferin A	Ashwagandha	SARS-CoV-2 (in silico)	Binds viral protease, modulates immune response	(Kumar et al.,2020)
Baicalein	*Scutellaria baicalensis*	SARS-CoV-2	Inhibits viral protease and RNA polymerase	(Su et al.,2020)
Ellagitannins	Pomegranate, berries	Norovirus, HSV	Binds viral capsid proteins	(Su et al.,2010)
Proanthocyanidins	Cranberries, grapes	Rotavirus, Norovirus	Inhibit viral binding and entry	(D'Souza et al.,2012)

Table 1 : Phytocompounds showing antiviral effect

2.7 PHYTOCOMPOUNDS IN *Ocimum sanctum* and *Withania somnifera* AS THERAPEUTIC AGENTS

2.9.1 Ocimum sanctum

India is well known to have spiritual and therapeutic values. Ocimum sanctum extracts are used for treating common cold, cough, and heart disease. The signature phytochemicals of the

Ocimum sanctum are: linalool, ursolic acid, rosmirinic acid, carvacrol etc. The secondary metabolite in the Ocimum sanctum includes:-

2.9.1.1 Phenolic compounds

These compounds are vital in disrupting viral structure boosts immune system to respond against the virus infection with antioxidant capability.They have phenolic aromatic rings with hydroxyl groups. It includes Eugenol, Rosmirinic acid, and cellular defense against oxidative damage while viral infection.

2.9.1.2. Flavonoids

Flavonoids are polyphenolic structures, behaves as natural antioxidants and modulates the immune system and suppresses the cytokine storm. They also regulate signalling pathways like MAPK and PI3K/Akt. It includes Apigenin and luteolin which stops the activity of Neuraminidase

2.9.1.3. Terpenoids/ Triterpenes

They are derived from isoprene units and they reflect anti-HIV, anti influenza effects and also show hepatoprotective effects. It disrupts the receptor interaction that leads to blocking of virus entry.

2.9.2 *Withania somnifera*

(Ashwagandha) ,most valued herb in traditional Ayurvedic and Unani medicine and members of the Solanaceae family, known to have adaptogenic qualities. It has a rich variety of secondary metabolite, especially withanolides, alkaloides and sitoindosides possess powerful antiviral, anticancer, anti-inflammatory properties. Especially withaferin A, withanolide A and withanone stand out for their antiviral potential and are now being explored for their effectiveness against viral infections. In this literature review phytocompounds from Withania somnifera were deeply studied and possess various secondary metabolites such as:-

2.9.2.1. Alkaloids

These are phytocompounds containing Nitrogen that often act on the nervous system. They can modulate neurotransmitory activities and help in reducing stress and inflammation. Alkaloid phytocompound from Withania somnifera include Somniferine, exhibiting hypotensive, antimicrobial, sedative effects. Tropine & cuscohygrine are tropane alkaloids affect the PNS

2.9.2.2. Withanolides(Steroidal Lactones)

Ashwagandha is known to have Withanolides including Withaferin A, Withanolide A, and withanone. Sitoidosides (VII- X) behave like adaptogens, enhancing the balance of the immune system. This class of phytocompound helps in reducing oxidative stress and regulates immune response.

2.8 ROLE OF MOLECULAR DOCKING AND ADMET PROFILING IN DRUG DISCOVERY

2.8.1 Molecular docking

Computational Methods like Molecular docking is perform to predict the binding Energy of macromolecular target like enzyme or protein and a small molecule called ligand. Molecular docking helps to identify how ligand interact with target's active site or binding site which helps to determine the binding affinity and stability of the complex. (Kitchen et al., 2004; Meng et al., 2011) . This technique is widely utilized in structure- based drug design as it mimics how a drug interact with its biological target (Ferreria et al., 2015) . This approach integrates both scoring function (to rank these compounds on the basis of their binding affinity) and search algorithms (to determine possible placement and conformarion) (Trott & Olson, 2010;Morris et al., 2009). Energy fluctuations are related to the binding constant (Kd) and the Gibbs Free Energy (GGL) in the course of the ligand-receptor model's development (Foloppe & Hubbard, 2006)

2.8.1.1 Key Concepts in Molecular Docking

- **Ligand and Receptor:** The ligand is usually a small, bioactive compound, while the receptor is a biological macromolecule like a protein. Docking explores how these interact in a specific binding site (Meng et al., 2011).

- **Binding Site Identification:** Determining where on the protein the ligand is likely to bind, often through structural data or prediction tools (Ferreira et al., 2015).

- **Pose Prediction:** The "pose" refers to the 3D orientation and conformation of the ligand when bound to the receptor (Kitchen et al., 2004).

- **Scoring Functions:** These are algorithms that evaluate how favorable a given ligand-receptor interaction is, based on factors like hydrogen bonding, hydrophobic interactions, and van der Waals forces (Trott & Olson, 2010).

- **Relevance in Drug Discovery:** Docking accelerates the identification of promising drug candidates by virtually screening compound libraries against disease-related targets (Pagadala et al., 2017). For example, Kumar et al. (2020) used molecular docking to predict that Withanone, a phytochemical from Withania somnifera, could inhibit the main protease (Mpro) of SARS-CoV-2.

2.8.2 ADMET Profiling

In order to identify a promising drug candidate which ensures efficacy and safety before clinical trials and reduces the cost and failure rate during advanced phase of drug developmentt, ADMET profiling is used. This helps to evaluate whether a phytocompound has required Pharmacokinetic and safety profile to be used as a potential drug molecules. Various tools can be utilized for ADMET profiling such as SwissADME, pkCSM, ADMETlab (In silico), cell culture assays(In vitro) and animal models(in vivo) in later

stages. Past studies prioritize advanced computation tools in identification of phytocompound with necessary Pharmacokinetic & safety profiles. SwissADME offers quick assesment to determine variables such as solubility, lipophylicity(Log P), P-gp, hydrogen bonding and polar surface area of a compound which influences gastrointestinal absorption and bioavailability of a compound (Wang,2017). Vd(Volume of distribution), plasma-protein binding, BBB permeability are the key parametees which describes about the distribution of drug throughout the body (blood, tissues, organs). Metabolism profiling involves how the drug is become active or inactive (usually in liver). Enzymes like Cytochrome P450 is involved. This helps in determining whether the drug has stable and harmful metabolites. Excretion profiling predict the elimination of drug and its metabolite from the body (kidneys, liver, lungs) and hepatotoxicity,cardiotoxicity (hERG channel inhibition), AMES toxicity enables to determine whether a drug has potential harmful effect(Daoud et al., 2021). Tools like pkCSM evaluate the drug toxicity. In SwissADME analysis ,Lipinski rule of five predict the drug-like properties of the compound Based on Molecular weight (MW) ≤500Da, LogP(≤5) , H- bond donor(≤5), H- bond acceptors(≤10) parameters. Combining computational tools with experimental data enhances the accuracy and safety of the drug like phytocompond with a higher probability of clinical success.

2.9 IN SILICO SCREENING OF PHYTOCOMPOUNDS AGAINST INFLUENZA VIRUS

Influenza being a contagious respiratory pathogen responsible for seasonal epidemics shows antigenic shift and antigenic drift, causing resistance to drugs like oseltamivir. So, traditional knowledge is integrated with advanced computional methods & tools like Molecular Docking, SwissADME and pkCSM . This strategy predict the safety, accuracy and drug likeness of potential drug candidate . Here is an overview of previously found potential drug candidate for influenza virus.

Plant Source/ Compound	Targeted Influenza Protein	Method used	Key Findings	References
Coleus amboinicus/ Isosalvianolic acid	Hemagglutin	Molecular docking, ADMET, Molecular dynamics	High binding affinity; stable protein-ligand complex	(Priya et al.2024)
Himalayan medicinal plants/ Clicoemodin, Rumexoside	Hemagglutinin and Nucleoprotein	Docking, Molecular dynamics	Dual-target potential; strong docking score; stabe complexes	(Sharma et al,2024)

Isatis tinctoria/ Various compounds	Influenza A viral Proteins	Molecular docking	Several compounds showed strong binding and inhibition potential	(Zhang et al.,2021)
Mangifera indica/ Mangiferin	Neuraminidase	Molecular docking	strong binding affinity; interaction with active site residues	(Rathore et al., 2022)
Camellia sinensis/EGCG	Neuraminidase	Molecular docking (Autodock Vina)	Form stable hydrogen bonds with NA active site; potential to inhibit viral replication	(Joshi et al., 2021)
Curcuma Longa/Curcumin	Hemagglutinin	Docking (Autodock Tools), MD Simulations	Strong binding at HA receptor-binding site; could block host cell entr	(Singh et al., 2019
Andrographis paniculata/ Andrographolide	Hemagglutinin	Docking(Glide), MM-GBSA	Form strong interactions with HA, MM-GBSA confirmed complex stability	(Rao et al., 2022)
Allium cepa/ Quercetin	Hemagglutinin	Molecular Docking, QSAR Analysis	Prevent HA mediated recognition to host sialic acid receptors	(Mehta et al.,2023)

Table 2: Phytocompounds from their plant sources against Influenza

CHAPTER 3

METHODOLOGY

3.1 RESOURCES

Various databases have been extensively utilized in this study to collect, analyze, and interpret relevant biological and chemical information. These are listed below:

3.1.1 PubMed:

Managed by NCBI and NIH, PubMed is a freely accessible database for literature studies, offering a vast collection of research articles, reviews, and clinical studies in the field of life sciences, medicine, and biotechnology.

3.1.2 PubChem:

Under the stewardship of NIH, PubChem is a comprehensive catalog of chemical substances and their activities. It offers extensive data on information about the structural characteristics, physical and chemical attributes, biological activities, safety, and toxicity profiles for compounds such as carbohydrates, lipids, proteins, and nucleotides.

3.1.3 IMPAAT 2.0 (Indian Medicinal Plants, Phytochemistry And Therapeutics): A curated database focusing on Indian medicinal plants, their phytochemicals, and associated properties such as physiochemical characteristics, pharmacokinetics, drug-likeness, ADMET profiles, and therapeutic relevance.

3.1.4 Protein Data Bank (PDB): It is a place where data is kept from high quality three dimensional form of biomolcules like proteins and nucleic acids. PDB is used in many things like in e-top models, loading models, and new ways to develop drugs.

3.2 SOFTWARE UTILIZED

3.2.1 Open Babel:

It is a known open-source tool that can convert different modes or ways to order chemicals. It can handle lots of chemical file types and often called as “the chemical toolbox that speaks many languages”.

3.2.2 PyRx:

A virtual scan tool that works as a group called pretty AutoDock and AutoDock Vina. It is easy to use and means do much in very short time possible to measure how well they match with the drug on protein that is the target.

3.2.3 Biovia Discovery Studio:

This tool got a lot of detail to understand how things fit or work together. It eyerytime takes two or three pictures to compare a mobile item, how it hooks a target in the body, and how it works to compare or model.

3.3 DATA EXTRACTION

In this work, phytochemicals were carefully taken out from the IMPPAT 2.0 database with the help of selecting plants in which they have been linked with any specific therapeutic property. Following therapeutic properties were taken into account: anticancer, antinociceptive, anti-inflammatory, antimalarial, antiallergic, neuroprotective, antidiabetic, antifungal, antimicrobial, antiulcer, antioxidant, antidiarrhoeal, immunomodulatory, antitumor, analgesic, antipyretic, antiplasmodic, antistaminic, antiproliferative, anthelmintic, astringent, anti-hyperglycaemic, anti-HIV, aphrodisiac, anticonvulsant, anti-osteoporotic and anti-cociceptive. From these plant species, specifically Withania somnifera and Ocimum sanctum, phytochemicals were collected taking into account their bioactivities as mentioned in the following list. Phytochemicals were screened further based on their high gastrointestinal (GI) absorption, acceptable bioavailability (0.55), and minimal CYP enzyme inhibition. Only those phytochemicals which presented a documented properties in IMPPAT were picked. A total of 60 phytochemicals were finalized (30 from each plant), each associated with at least one of the above therapeutic properties

3.4 PROTEIN PREPARATION:

The 3D crystal structure of N2- neuraminidase(PDB ID:6BR5) is downloaded from PDB in

.pdb format. It is visualized and validated using Biovia Discovery studio software where water molecule and hetatom is removed & hydrogen added in polar form . then The files are saved in the (.pdbqt format). The receptor & ligand grid map has been created with the following cordinate : X= 25; Y= 25; Z = 25, and the center X = -33.7663; Y = 22.7749; Y =1.8396.

3.5 LIGAND PREPARATION:

The 3D structures of the ligands are downloaded from PubChem in the SDF format (https://pubchem.ncbi.nlm.nih.gov/).Once the ligands are downloaded from Pubchem they are converted into pdbqt using the PyRx that contain openBabel.

3.6 DOCKING USING Pyrx:

Molecular docking is a computer technique that predicts how well ligands will attach to receptor proteins. Docking is done with PyRx. It is a molecular docking tool that is openly accessible. Compared to other docking systems, it was thought to be Easy to use and open

source molecular Docking Tool .it has graphical user interface for AutoDock Vina and AutoDock. It not requiring command-line usage.It set Grid box for docking quickly and provides docking results. Docking parameters are configured via graphical user interface. target protein is loaded and converted into macromolecule. Subsequently, ligand is also loaded and their energy is minimized and converted into .pdbqt in open babel of pyrx software. Then for specific docking area of the grid represented on the software in the image is set according to specif centre XYZ. Then proceeded for docking.

Once Docking is done. a log file is generated showing the docking results and binding Affinity An out.pdbqt file containing the binding mode/pose. When the docking process is complete, a list of the best binding molecules is generated based on the best Binding Affinity, and the Best molecule is recommended for further study. The reference molecule, osaltamivir
, has a Binding Energy of 6.5 kcal/mol.

3.7 PROTEIN-LIGAND INTERACTIONS ANALYSIS

After the docking process was completed, all of the target-ligand interaction structures were captured in the out.pdbqt file and converted to PDB format. To evaluate every encounter, BIOVIA Discovery Studio (version v25.1.0.24284) was used.

3.8 PHARMACOKINETIC AND TOXICITY PREDICTION (ADME/PkCSM)

SwissADME (http://www.swissadme.ch/), an open-access online software, was used for analyzing pharmacokinetic properties of compounds. ADME analysis is used for each of the 14 phytochemicals that were selected from 60 drug candidate. The main criteria used in the evaluation was water solubility, lipophilicity, high gestrointestinal Absorption, Blood-brain barrier(BBB) permeability, violations of Rule of Lipinski, & Bioavailability.

Toxicity profiling was conducted using PkCSM, focusing on hepatotoxicity, carcinogenicity, and LD_{50} class prediction to assess safety and tolerability.

CHAPTER 4

RESULT

When 60 phytocompounds from medicinal plants evaluated were docked against Neuraminidase protein, the result showed a wide range in binding energies. The reference compound, Oseltamivir, had binding affinity of -6.5 kcal/mol. Some phytocompounds interact loosely with binding site of the target protein, while others interact tighltly. Only 11 phytocompounds were selected out of 60 compounds, having binding energy spanning from -9.2 to -7.5 kcal/mol. After that these 11 phytocompounds were analysed in SwissADME by using SMILE of these phytochemical to predict their Molecular weight, Lipinski violation, lopP value, Bioavailability, BBB, GIA, TPSA, H donor and H Acceptor. pkCSM was used to predict the toxicity of these compounds.

4.1 MOLECULAR DOCKING AND ANALYSIS

Pubchem CID	Common name	Plant source	Binding Energy(kcal/mol)
265237	Withaferin A	Withania somnifera(Ashwagandha)	-9.2
21679035	Withanone	Withania somnifera(Ashwagandha	-8.7
10494	Oleanolic acid	Ocimum sanctum(Tulsi)	-8.3
64945	Ursolic Acid	Ocimum sanctum(Tulsi)	-8.2
5281605	Baicalein	Ocimum sanctum(Tulsi)	-8.1
101537504	Withasomidienone	Withania somnifera(Ashwagandha)	-7.9
53178380	Vicenin-2	Ocimum sanctum(Tulsi)	-7.6
11294352	Withanolide A	Withania somnifera(Ashwagandha)	-7.6
5281676	Isoorientin	Ocimum sanctum(Tulsi)	-7.5
5280443	Apigenin	Ocimum sanctum(Tulsi)	-7.5
65028	**Oseltamivir**	**Synthetic (antiviral drug)**	**-6.5**

Table 3 : Result of phytocompounds showing binding in kcal/mol

Compound CID	Common name	Binding energy	Interacting Residues
265237	Withaferin A	-9.2	GLU A:277, ARG A:152, SER A:247, ASN A:294, HIS A:347
21679035	Withanone	-8.7	TYR A: 406,ARG A: 152
10494	Oleanolic acid	-8.3	ASN A:294, GLU A:227
64945	Ursolic Acid	-8.2	ASN A-294, GLU A: 227
5281605	Baicalein	-8.1	GLU A: 227,GLU A: 277, TRP A: 178, ASP A: 151, ARG A: 152, ALA A: 246, ILE A: 222
65028	**Oseltamivir**	**-6.5**	ARG A:118, ARG A:152, ARG A:224, ARG A:292, ARG A:371, GLU A:119, ASP A:151, TYR A:406, HIS A:347, ALA A:246, ILE A:222

Table 4 : Top 5 phytocompound with lowest binding energy and Interacting residues

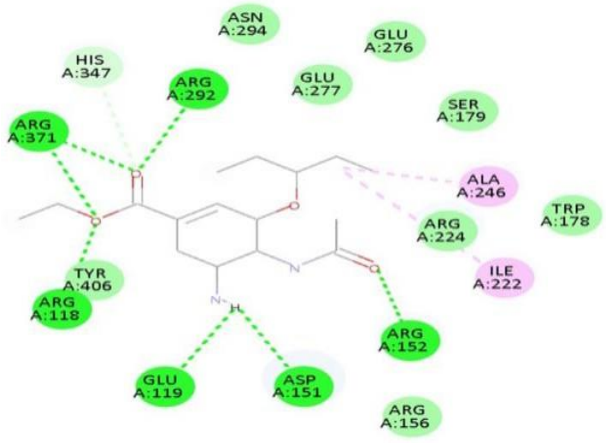

Figure 3 : Elucidation of the binding interaction between Oseltamivir (PubChem CID: 65028) and Neuraminidase in 2-D

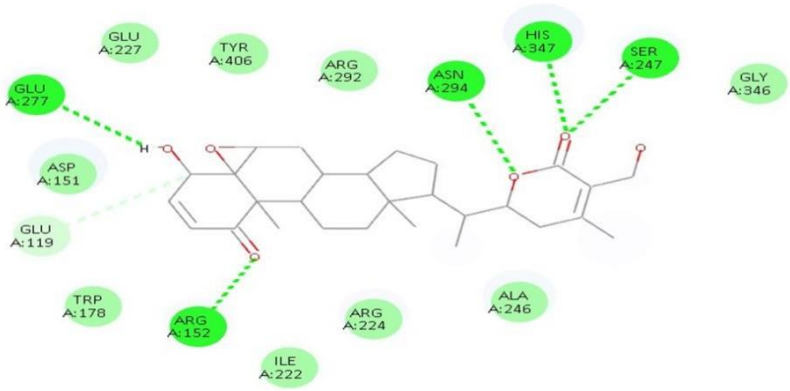

Figure 4 :Elucidation of the binding interaction between Withaferin A (PubChem CID:265237) and Neuraminidase in 2-D

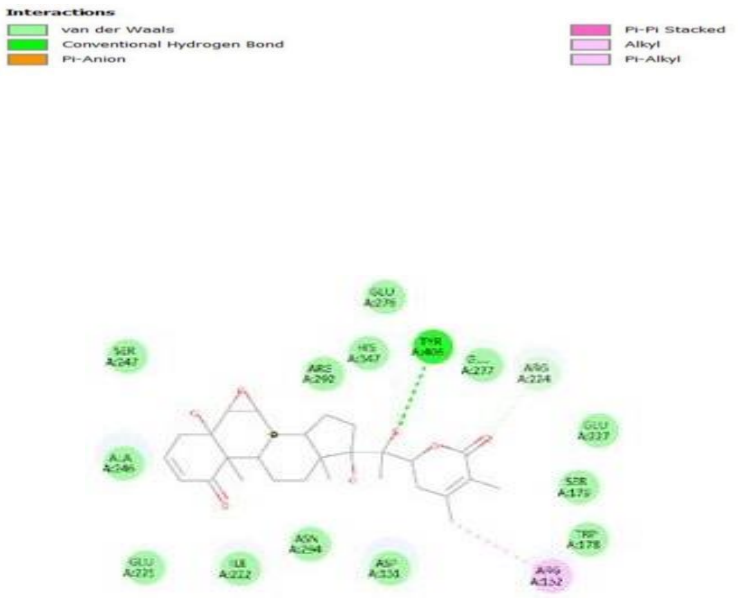

Figure 5 :Elucidation of the binding interaction between Withanone (PubChem CID: 21679035) and Neuraminidase in 2-D

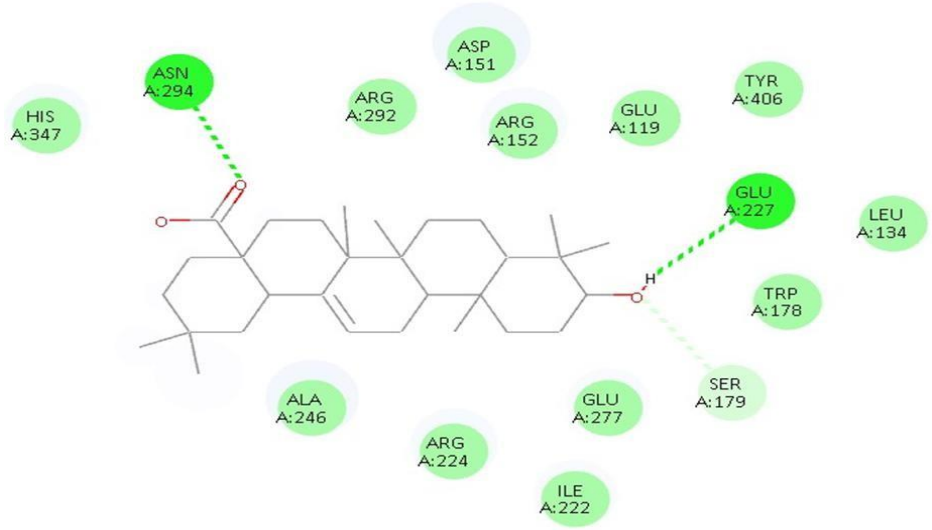

Figure 6 : Elucidation of the binding interaction between Oleanolic acid (PubChem CID: 10494) and Neuraminidase in 2-D

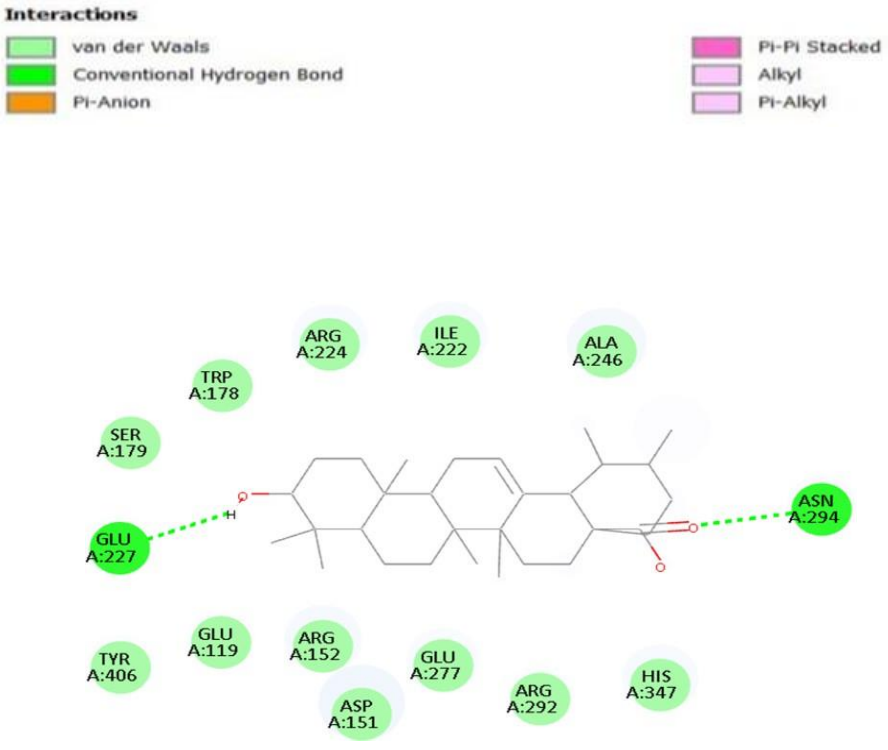

Figure 7 :Elucidation of the binding interaction between Ursolic Acid (PubChem CID: 64945) and Neuraminidase in 2-D

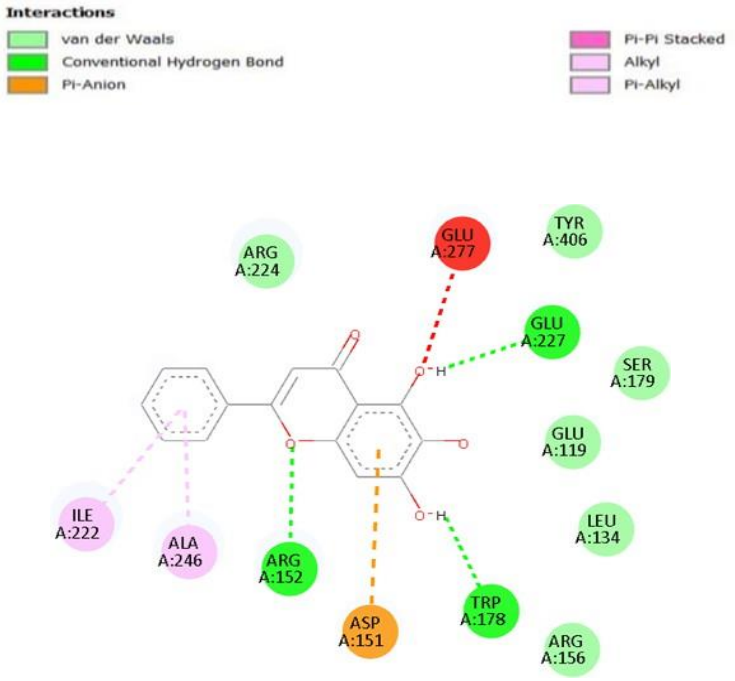

Figure 8 :Elucidation of the binding interaction between Baicalein (PubChem CID: 5281605) and Neuraminidase in 2-D

4.2 SwissADME RESULT :

phytocompound	Mol. Wt.	Lipinski violation	log P	Bioavailability	BBB	GIA	TPSA	H Donor	H Acceptor
Oseltamivir	312.40	0	1.38	0.55	No	high	90.65	2	5
Withaferin A	470.60	0	3.42	0.55	No	high	96.36	2	6
Withanone	486.60	0	2.53	0.55	No	high	116.59	3	7
Oleanolic acid	456.70	1	6.07	0.85	No	low	57.53	2	3
Ursolic Acid	456.70	1	5.93	0.85	No	low	57.53	2	3
Baicelein	270.24	0	2.24	0.55	No	high	90.90	3	5
Withasomidienone	468.55	0	4.59	0.55	No	high	61.42	0	4
Vicenin-2	330.42	0	3.43	0.55	Yes	high	63.60	1	4
Withanolide A	470.37	0	4.04	0.55	No	high	89.13	0	6
Isoorientin	360.31	0	2.00	0.55	No	high	118.59	3	8
Apigenin	270.24	0	2.11	0.55	No	high	90.90	3	5

Table 5 : ADME Results from SwissADME

4.3 PkCSM RESULT:

Common name	Ames's toxicity	hERG I inhibitor	hERG-II inhibitor	Lethal Dose 50 (LD50)	Hepatotoxicity
Withaferin A (265237)	No	No	No	2.779	No
Withanone (21679035)	No	No	No	2.779	No
Oleanolic acid (10494)	No	No	No	2.779	No
Ursolic Acid (64945)	No	No	No	2.346	Yes
Baicalein (5281605)	No	No	No	2.779	No
Withasomidienone (101537504)	No	No	Yes	3.029	Yes
Vicenin-2 (53178380)	No	No	Yes	3.167	Yes
Withanolide A (11294352)	No	No	No	2.478	No
Isoorientin (5281676)	No	No	No	2.315	No

Table 6 : Toxicity result from PkCSM

CHAPTER – 5

CONCLUSION AND DISCUSSION

In conclusion, the Aim of our study was to Screening & identify a potential phytocompound with antiviral action Against Oseltamivir drug resistant strains of Influenza virus which is safer to use in the body. Indian medicinal plants like Ocimum sanctum ,curcumin longa, Withania omnifera are well known for their therapeutic action . For years, plant based treatment has been a topic of interest in drug research and development . These plant based compounds are biocompatible and less harmful to body, while synthetic drugs are chemically synthesized and can have side effects. They serve as a reservoir of these secondary metabolites having promising therapeutic effect. Researchers have gained in-depth insights into the complex chemicals of the plant by utilizing modern computational(in silico) approaches. Their results have lay the groundwork for further trials and extended their utility in biotechnological research and other discipline. Without integrating modern tool with traditional knowledge ,it became very difficult to predict the possibility of bio-active compounds to serve as treatment option, immunomodulation or nutrient supplementation.In this study Withaferin A (Pubchem ID : 265237) from Withania somnifera showed strong binding affinity(-9.2 kcal/mol) to NA and suitable pharmacokinetic properties based on ADMET profiling. It exhibited no Lipinski rule violation, high GI absorption, acceptable bioavailability(0.55) and minimal CYP enzyme inhibition. Additionally, it was non- hepatotoxic and non-mutagenic. These results indicate its potential as lead candidate for drug development. In silico results have lay the groundwork for further trials and extended their utility in biotechnological research and other discipline. As these results are based on in silico estimation and hence need experimental validation.

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PUBLICATION

Title of Paper - "In-silico Investigation Of Phytocompounds From *PolyGonum cuspidatum* As Therapeutic Drug Candidate For 1F9F Protein Of Human Papillomavirus(HPV)

Author Names – Pinki, Ravishankar Kumar, Navneeta Bharadvaja

Name of Conference –Second International Conference on Emerging Technologies in Science and Engineering (ICETSE)-2025 organised by the Akshaya Institute of Technology and technically co-sponsored by Hinweis Research.

Date of Conference – June 19-20, 2025

Indexing - Scopus Indexed

Status of Paper – Acceptance Received

Date of Acceptance – May 06, 2025

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ICETSE 2025 <icetse2025@gmail.com> Tue, 6 May, 2025 at 6:42 pm
To: Ravishankar Kumar <ravishankarkumar0705@gmail.com>

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On Tue, 6 May, 2025, 1:56 pm Ravishankar Kumar, <ravishankarkumar0705@gmail.com> wrote:

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> I, Ravishankar kumar (Roll no. 23/MSCBIO/41) and Pinki (23/MSCBIO/37) are 2nd-year MSc Biotechnology students at Delhi Technological University. We would like to submit our conference paper titled " " in your esteemed conference.
> Due to a technical issue, we were unable to get our paper submitted by the due date. I kindly request that you take our problem into consideration and accept our submission. I am attaching the full length ready paper below.
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> Thanking you

Re: Payment and registration form
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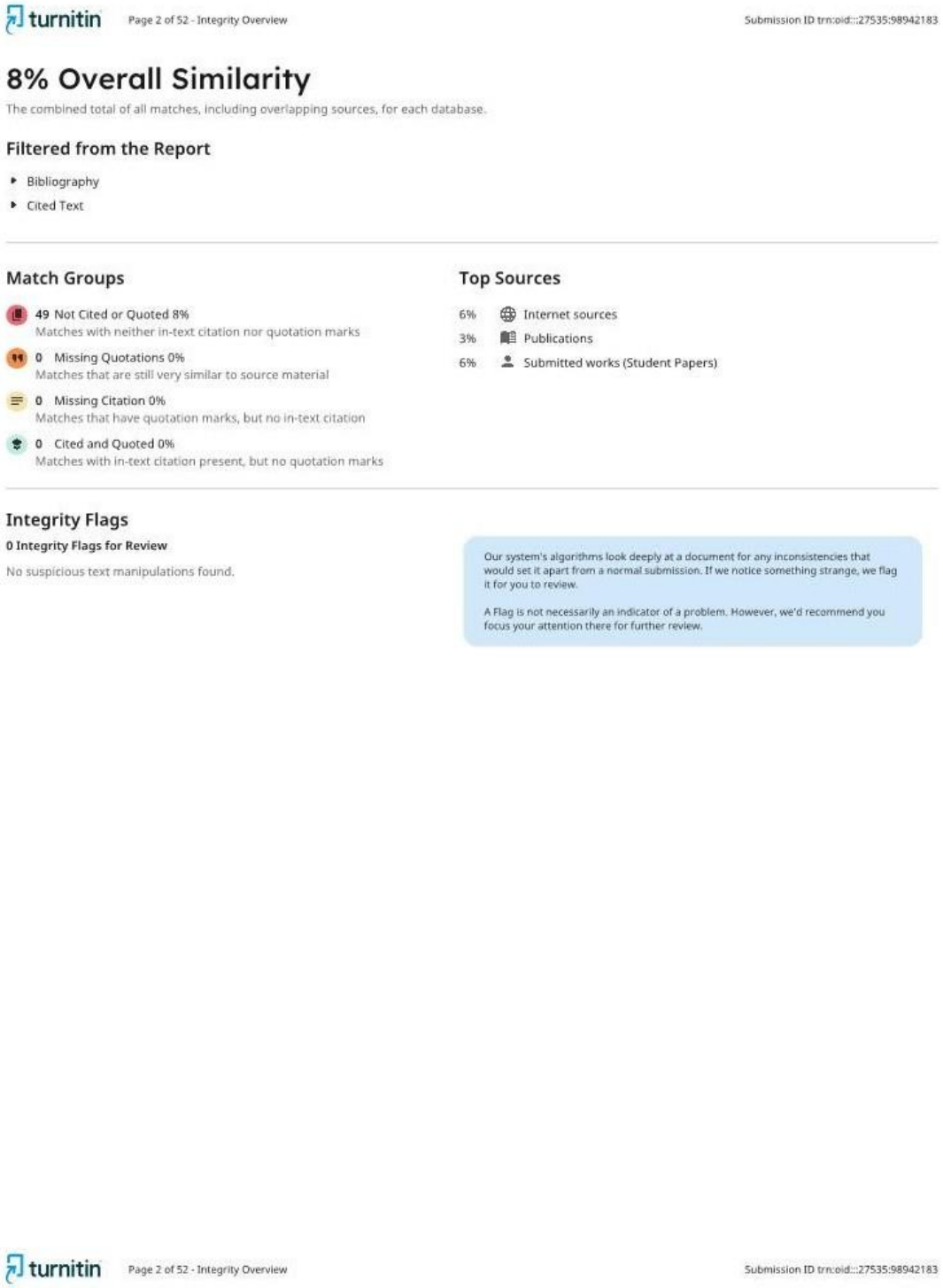

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