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Structure based Computational Investigation of Phytocompounds from Vitex Negundo as Drug Candidate for Human Respiratory Syncytial Virus (hRSV)

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***Abstract*— Human Respiratory Syncytial Virus (hRSV) is the primary factor contributing to illness in adults and death in infants on a global scale. All throughout life, recurring infection only provides transient immunity and remains a major cause for the development of both cardiovascular and respiratory complications. Ribavirin is the sole FDA-approved vaccination for severe RSV illnesses, but its usage is restricted due to side effects, high cost, and a complex administration method. Therefore, drugs targeting the virus is an urgent demand in the current scenario. The therapeutic active substances present in Vitex negundo, a medicinal plant can potentially serve as a basis for the synthesis of herbal drugs. In this study molecular docking was carried out and the prominent active compounds found in the plant were analyzed on hRSV which indicated excellent interactions with appropriate binding affinities. Isovitexin outperformed all other bioactive compounds in terms of binding energy of -8.7 kcal/mol, making it the most potential bioactive compound to be used as drug target.**

***Index Terms*— Vitex negundo, Human Respiratory Syncytial Virus, Bioactive compounds, In silico, Docking.**

I. INTRODUCTION

The Human Respiratory Syncytial Virus (hRSV) especially infects infants and children and also the elderly, and immunocompromised people. It causes lower respiratory tract infections (LRTI), which includes both pneumonia and bronchitis. It belongs to the Paramyxoviridae family and the Mononegavirales order. The genome of this virus is made up of a single-stranded RNA that is about 15.2 kb long and is surrounded by a protein capsid [6]. The genome of RSV encodes 11 proteins out of which 3 are viral envelope proteins. Fusion glycoprotein is one of the envelope proteins and is responsible for facilitating viral entry [2]. The fusion glycoprotein F of hRSV is an integral membrane protein of type I derived from a 574-amino acid precursor. A Furin-like protease cleaves the 27-residue glycosylated peptide (pep27) into the F2 (N-terminal region) and F1 (C-terminal region) polypeptides. Two disulfide connections unite these subunits to form a protomer, which then polymerizes to become the mature trimeric F protein [8]. F glycoproteins undertake a conformational change during the entry of cell that brings the cell membrane of the virus and host into close proximity, eventually leading to their fusion and formation of an exceptionally stable post-fusion F protein conformation [9]. A favourable technique for combating severe RSV

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involves inhibiting viral fusion using targeted antiviral drugs. The primary antigenic target used in vaccine development for RSV is the F protein. Palivizumab is the sole authorized monoclonal antibody that focuses on the conserved epitope in the F protein of the viral envelope. This neutralizing antibody has been proven to effectively decrease the severity of the condition. RSV bronchiolitis has a typical characteristic of mucus in the airways, an abundance of neutrophils, and sloughed epithelial cell debris. RSV lower respiratory tract infection is associated with airway mucus, which can cause pulmonary obstruction. However, the mechanisms causing mucus expression are unknown [6]. As a global health priority, RSV is becoming more widely acknowledged. The main manifestation of RSV LRTI is airway mucus, which exacerbates pulmonary obstruction. Cardiopulmonary and respiratory complications are developed as a result of RSV, which continues to be a leading cause of mortality. The number of RSV cases in children under five is estimated to be 33 million worldwide each year, with 11% of those cases resulting in hospitalization [5]. In Spain between 2016-2019, highest hospitalization was observed among children under 5 years of age for respiratory diseases linked to RSV while in adults, ≥ 80 years hospitalization was observed for cardiorespiratory diseases linked to RSV [4]. Ribavirin, the only licensed vaccine against RSV is a synthetic nucleoside with a significant antiviral activity. Numerous studies have been carried out for the evaluation of the efficacy of ribavirin against RSV. Ribavirin therapy used early in medical intervention may lower RSV death rates in young children [3]. Since ancient times, people have utilized herbal medicines as a primary source of healthcare. Its history is as old as humanity itself. The therapeutic active substances found in the traditional medicinal plants serve as a basis for the synthesis of herbal drugs. *Vitex negundo* is a medicinal plant which is a member of the Verbenaceae family. Due to the presence of various therapeutic active compounds in the plant it is widely used in the treatment of jaundice, cancer, liver disorder, and asthma [7]. Some of the prominent bioactive compounds of *Vitex negundo* will be considered for further analysis on RSV. Performing in silico analysis using molecular docking on these compounds will help us comprehend fundamental biochemical processes and describe the interaction of the bioactive compounds within the binding sites of the target protein. The optimal configuration of the receptor-ligand complex molecule will be determined through this molecular docking.

II. Materials And Methods

A. Data Collection

For the current work, we employed bioinformatics tools and biological databases such as PubMed, Protein Data Bank (PDB), IMPPAT, PubChem, as well as software such as Biovia Discovery Studio, PyRx, PyMol, PLIP and Swiss ADME.

B. Protein Structure Retrieval and Preparation

A 2.50 Å resolution of the 5KWW (F protein) 3D crystal structure was downloaded. The RCSB PDB was used for this (https://www.rcsb.org/). The protein was initially unsuitable for molecular docking in its current state. It was imported into Biovia Discovery Studio to eliminate water molecules, ligands, and heteroatoms. This process aimed to improve the precision of projected electrostatic interactions and stabilise the protein-ligand interactions. These components are not essential to the protein and can disrupt the attachment of specific ligands to the target. They are frequently eliminated to enhance the precision and effectiveness of the docking calculations. The protein that was produced was stored in PDB format.

C. Constructing a Ligand Library

The three-dimensional configuration of 19 active phytochemicals found in Vitex negundo namely Vitexin, Isovitexin, Negundoside, Kaempherol, Luteolin, Isoorientin, Bergenin, Agnoside, Artemetin, Idopyranoside, L-Linalool, Pumilin, Beta-carotene, Friedelanol, Chrysosplenol D, Chrysosplenetin, Vitexicarpin, Vitexilactone, and Vitedoin A were downloaded from PubChem in SDF format.
The 3D conformer of Emodin, a widely recognised model inhibitor of 5KWW utilised as a control, was obtained in SDF format from PubChem.

D. Molecular Docking and Analysis

Molecular Docking was conducted using the PyRx software containing AutoDock Vina module available at https://pyrx.sourceforge.io/. The program accepts protein in PDB format and ligand in SDF format. The target protein was docked with the Emodin inhibitor as a control, and the binding energy was calculated. After the completion of the process, a 2D diagram of the protein-ligand interaction was generated. The target protein was docked with 19 bioactive chemicals from Vitex negundo samples using the same procedure. The result file was

loaded into PyMol (https://pymol.org/2/) together with the receptor protein to create a complex for analyzing the interaction between the protein and ligands in 3D space. The PDB format of the complex was then stored and analyzed using PLIP to examine the crucial interactions between the protein and the ligand.

E. ADME Analysis

The effectiveness, harmful effects, and how the body processes the suggested bioactive chemicals were evaluated by obtaining the sequence of each compound in SMILE format from IMPPAT and fed into the online tool, Swiss ADME (http://www.swissadme.ch/), which determined whether or not the selected phytocompounds would be good candidates for drug research which is crucial for regulatory approval.

III. RESULTS

A. Molecular Docking

Plant-based medicines and computer-aided drug design are both regarded excellent methods for discovering and developing drugs. Computational drug development extensively uses in silico techniques, molecular docking, and molecular dynamic simulations to identify new compounds [2]. Emodin, which is considered as a model inhibitor of Fusion glycoprotein (5KWW) of RSV showed a binding affinity of -7.5 Kcal/mol. It exhibits 1 unfavourable donor-donor interaction, 2 pi-donor hydrogen bonds, 2 alkyl bonds, 2 pi-sigma bonds, and 6 Van der Waals interactions while binding to RSV's F glycoprotein i.e. 5KWW as shown in Fig 1.

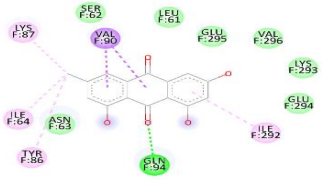


Figure 1. 2D interaction of Emodin with 5KWW

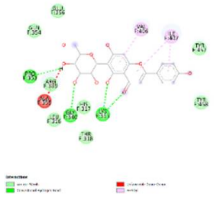
Analysis of several phytocompounds was done by comparing their binding strengths and studying the interaction between the protein and ligand and it was found that Isovitexin showed a binding affinity of -8.7 Kcal/mol which shows a better degree of interaction which in turn corresponds to better inhibition of the protein which will inhibit the viral entry in the body. The top three hits, Isovitexin, Isoorientin, and Beta-carotene, presented high affinity bound to 5KWW with docking affinity of -8.7 kcal/mol. The phytocompounds which show the minimum binding affinity can be further considered for analysis as drug targets.

All the phytocompounds used for analysis are mentioned in Table I along with their respective binding affinities in decreasing order. Fig 2 depicts the 2D structure of all the protein-ligand interactions as downloaded by Biovia Discovery Studio.

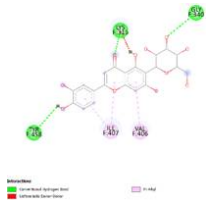
TABLE I. ESTIMATED BINDING ENERGIES

Bioactive compounds	**Binding Affinity**
Isovitexin **(A)**	-8.7
Isoorientin **(B)**	-8.7
Beta-carotene **(C)**	-8.7
Friedelanol **(D)**	-8.4
Luteolin **(E)**	-7.4
Agnoside **(F)**	-7.4
Kaempherol **(G)**	-7
Pumilin **(H)**	-7
Negundoside **(I)**	-7
Vitexicarpin **(J)**	-6.7
Berginin **(K)**	-6.7
Crysosplentin **(L)**	-6.6
Vitexilactone **(M)**	-6.5
Crysosplenol D **(N)**	-6.5
Vitexin **(O)**	-6.4
Artemetin **(P)**	-6.4
Vitedoin A **(Q)**	-6.2
Idopyrinoside **(R)**	-5.1
L-Linalool **(S)**	-4.9

The interactions yielded hydrogen bonds with LYS315, GLY340, TYR457, PRO353, ARG339 residues and pi-alkyl bonds with VAL406 and ILE407 residues. In addition to these, Van der Waals interactions and donor-donor interactions were also observed.



(A)



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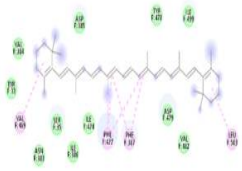

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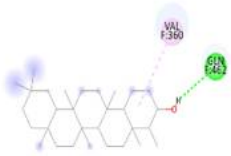

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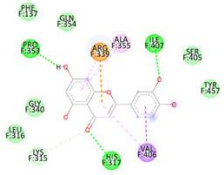

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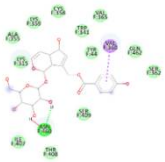

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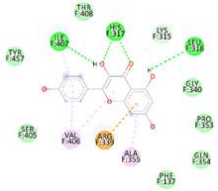

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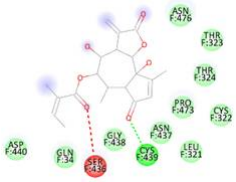

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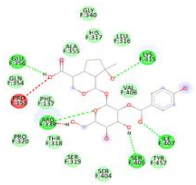

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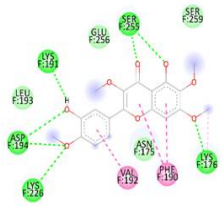

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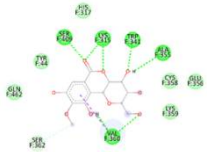

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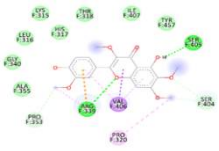

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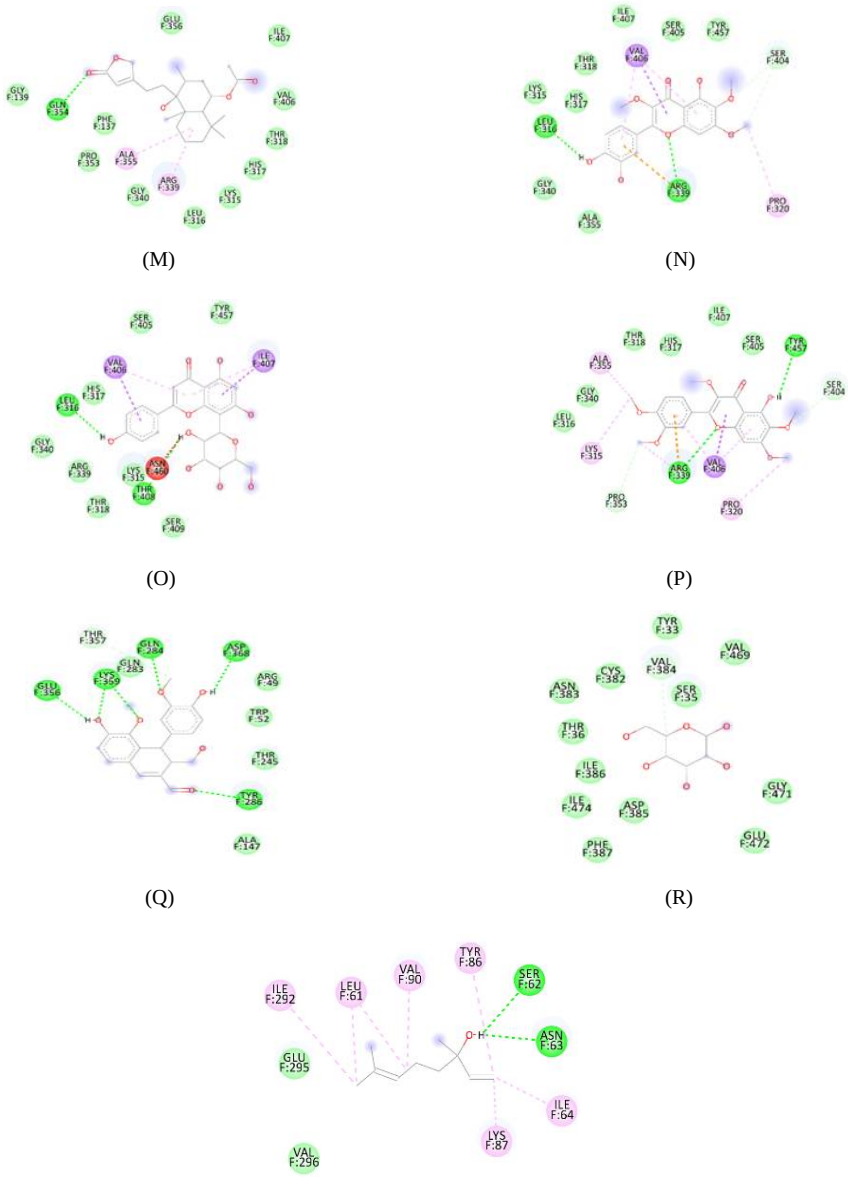

Figure 2. Interaction of the selected phytocompounds with 5KWW (target protein)

B. ADME Properties

Pharmacokinetic analysis is done to identify whether the test compounds can potentially act as drug molecules by assessing the presence of drug likeliness properties. To achieve so, the assessments were done by SWISS ADME [3]. The SWISS ADME analysis revealed that the majority of phytocompounds have a moderate bioavailability score of between 0.55 or 0.56. These study the pharmacokinetic characteristics of a drug by analyzing its absorption, metabolism, distribution, and excretion processes. All selected bioactive compounds have a molecular weight of less than 500 Dalton (g/mol). Drugs having molecular weights below 500 g/mol can pass through the skin and blood vessels [1].

Figure 2 illustrates RADARS (Ranking Aggregated Drug Attributes by Relevance and Similarity) which shows the bioavailability of the first three phytocompounds. These illustrate the pharmacological properties of the suggested substances. These are generated using computational approaches and algorithms that consider several physiochemical factors like size, flexibility, saturation, lipophilicity, and polarity. The pink region represents the standard range for every characteristic.

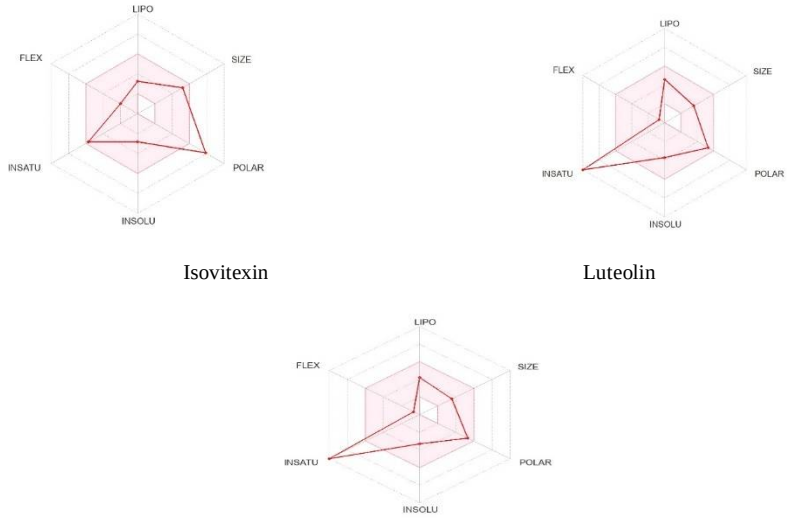

Figure 2. Radar plots of Isovitexin, Luteolin and Kaempherol

V. Conclusion

RSV can be inactivated by treatments that target retroviruses' F glycoprotein, as shown in this study. In the last 15 years', there have been many discoveries of small-molecule fusion inhibitors. While many of these agents have demonstrated strong inhibitory effects against RSV, their development has been hindered by unfavorable drug distribution in the body or a limited safety profile. As a result, only a small number of molecules are currently undergoing clinical trials. All of the bioactive chemicals used in this study inhibited RSV, and their natural occurrence, oral bioavailability, and non-toxic nature, as demonstrated by the ADME analysis, make them extremely safe to use. Isovitexin outperformed all other bioactive compounds in terms of binding energy. Isoorientin and Beta-carotene both show good outcomes. The current exploratory molecular docking study could be a big step forward in experimentally establishing their antiviral nature and mechanism, as well as a valuable research tool for better understanding the immunobiology of RSV disease.

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References

[1] Bos, J. D., & Meinardi, M. M. H. M. (2000). The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Experimental Dermatology, 9(3), 165–169. https://doi.org/10.1034/j.1600-0625.2000.009003165.x.
[2] Dali Y, Abbasi SM, Khan SAF, Larra SA, Rasool R, Ain QT, Jafar TH. Computational drug design and exploration of potent phytochemicals against cancer through in silico approaches. Biomedical Letters 2019; 5(1):2126.
[3] Foloppe, N., & Hubbard, R. (2006). Towards predictive ligand design with free-energy based computational methods. Current medicinal chemistry, 13(29), 3583–3608. https://doi.org/10.2174/092986706779026165.
[4] Graham, B. S., Modjarrad, K., & McLellan, J. S. (2015). Novel antigens for RSV vaccines. In Current Opinion in Immunology (Vol. 35, pp. 30–38). Elsevier Ltd. https://doi.org/10.1016/j.coi.2015.04.005
[5] Groothuis, J. R., Woodin, K. A., Katz, R., Robertson, A. D., Mcbride, J. T., Hall, C. B., Mcwiiliams, B. C., & Lauer, B. A. (n.d.). PEDIATRIC PHARMACOLOGY AND THERAPEUTICS Early ribavirin syncytial viral children treatment of respiratory infection in high-risk.
[6] Haeberer, M., Bruyndonckx, R., Polkowska-Kramek, A., Torres, A., Liang, C., Nuttens, C., Casas, M., Lemme, F., Ewnetu, W. B., Tran, T. M. P., Atwell, J. E., Diez, C. M., Gessner, B. D., & Begier, E. (2024). Estimated Respiratory Syncytial Virus-Related Hospitalizations and Deaths Among Children and Adults in Spain, 2016–2019. Infectious Diseases and Therapy. https://doi.org/10.1007/s40121-024-00920-7.

[7] Li, Y., Wang, X., Blau, D. M., Caballero, M. T., Feikin, D. R., Gill, C. J., Madhi, S. A., Omer, S. B., Simões, E. A. F., Campbell, H., Pariente, A. B., Bardach, D., Bassat, Q., Casalegno, J. S., Chakhunashvili, G., Crawford, N., Danilenko, D., Do, L. A. H., Echavarria, M., … Nair, H. (2022). Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. The Lancet, 399(10340), 2047–2064. https://doi.org/10.1016/S0140-6736(22)00478-0.

[8] Meng, J., Stobart, C. C., Hotard, A. L., & Moore, M. L. (2014). An Overview of Respiratory Syncytial Virus. PLoS Pathogens, 10(4), e1004016. https://doi.org/10.1371/journal.ppat.1004016.

[9] Pawar, S., & Kamble, V. (2017). PHYTOCHEMICAL SCREENING, ELEMENTAL AND FUNCTIONAL GROUP ANALYSIS OF VITEX NEGUNDO L. LEAVES. International Journal of Pharmacy and Pharmaceutical Sciences, 9(6), 226. https://doi.org/10.22159/ijpps.2017v9i6.18093.

[10] Souza, C., Zanchin, N. I. T., Krieger, M. A., & Ludwig, A. (2017). In silico analysis of amino acid variation in human respiratory syncytial virus: Insights into immunodiagnostics. Memorias Do Instituto Oswaldo Cruz, 112(10), 655–663. https://doi.org/10.1590/0074-02760170013.

[11] Swanson, K. A., Settembre, E. C., Shaw, C. A., Dey, A. K., Rappuoli, R., Mandl, C. W., Dormitzer, P. R., & Carfi, A. (2011). Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers. Proceedings of the National Academy of Sciences, 108(23), 9619–9624. https://doi.org/10.1073/pnas.1106536108.