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IN-SILICO DOCKING ANALYSIS OF PHYTOCHEMICALS FROM *VERBASCUM PHLOMOIDES* L. AS AN ANTIVIRAL AGENTS AGAINST HERPES SIMPLEX VIRUS TYPE I AND TYPE II

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ABSTRACT: Almost 85% of the world population is infected with the Herpes Simplex Virus (HSV). Herpes simplex virus infections have been described in the medical literature for centuries, yet the drugs available nowadays for therapy are largely ineffective and low oral bioavailability plays an important role on the inefficacy of the treatments. Additionally, the details of the inhibition of Herpes virus type 1 are still not fully understood. The present study was undertaken to evaluate the antiviral property of selected phytochemicals from *Verbascum phlomoides* L. through their interaction with the Herpes simplex virus. Depending on the non-covalent interaction and best docking score it was concluded that these phytochemicals may be useful for treatment against Herpes infection and can act as inhibitors to Herpesviral protein receptor. Also using phytochemicals as a medium of medication may reduce the cost dependent factor and can be widely used as medicinal purposes to treat several chronic and dreadful diseases.

INTRODUCTION: A virus is a particle of DNA or RNA enclosed by structural proteins. Viruses infect cells and proliferate to cause viral infections. Clinical features classify viral skin diseases into three types: degeneration of epidermal cells and blistering, tumorous changes in epidermal cells, and allergic eruptions on the whole body [1]. The Herpesviridae family is one of the major viral families. It includes 100 identified viruses that affect almost all animal species [2].

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Herpesviridae is subdivided into three subfamilies- Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. Simplex virus (HSV-1 and -2) belong to Alphaherpesvirinae. Herpes simplex viruses are ubiquitous, host-adapted pathogens that cause a wide variety of disease states. There are 9 types of human herpes viruses namely herpes simplex viruses types 1 and 2, varicella zoster virus, Epstein–Barr virus, cytomegalovirus, roseoloviruses HHV-6 (A and B) and HHV-7, and Kaposi sarcoma-associated herpesvirus (HHV-8) [3, 4] from which only two are selected for this study- HSV 1 and HSV 2.

Both are closely related but differ in epidemiology. HSV-1 is traditionally associated with orofacial disease, while HSV-2 is traditionally associated with the genital disease.

The term herpes is derived from the Greek word "to creep or crawl." Up to 80% of herpes simplex infections are asymptomatic. Symptomatic infections can be characterized by significant morbidity and recurrence. The prevalence of HSV infection worldwide has increased over the last several decades, making it a major public health concern. Prompt recognition of herpes simplex infection and early initiation of therapy are of utmost importance in the management of the disease. HSV is an enveloped virus that is approximately 160 nm in diameter with a linear, double-stranded DNA genome. The overall sequence homology between HSV-1 and HSV-2 is about 50%. HSV-1 has tropism for oral epithelium, while HSV-2 has tropism for genital epithelium. HSV infection is mediated through attachment via ubiquitous receptors to cells, including sensory neurons, leading to the establishment of latency [5].

HSV is transmitted by close personal contact, and infection occurs *via* inoculation of virus into susceptible mucosal surfaces (*e.g*., oropharynx, cervix, conjunctiva) or through small cracks in the skin. The virus is readily inactivated at room temperature and by drying; hence, aerosol and fomitic spread are rare [6].

Worldwide rates of either HSV-1 or HSV-2 are between 60 and 95% in adults. HSV-1 is more common than HSV-2, with rates increasing as people age. HSV-1 rates are between 70% and 80% in populations of low socioeconomic status and 40% to 60% in populations of improved socioeconomic status. Prevalence of HSV-2 in those between the ages of 15 and 50 is about 535 million as of 2003 or 16% of the population, with highest rates in sub-Sahara Africa and lowest in Western Europe, and with greater rates among women and those in the developing world. In the U.S, 57.7% of the population is infected with HSV-1 and 16.2% are infected with HSV-2, and the prevalence of HSV-2 was 39.2% in blacks and 20.9 % in women. More than 3.7 billion people under the age of 50 or 67% of the population are infected with herpes simplex virus type 1, according to WHO's global estimates of HSV-1 infection published on 28 October 2015. [7]

Locally, data analysis of sexually transmitted diseases was carried out to study the pattern of these diseases. One thousand five hundred and seventy-one patients were seen from January 1977 to October 1985. Males constituted 95.5% of this group and females the remaining 4.5%. Commonest age group affected was 20-29 years in both sexes. The rate of the population affected by genital herpes was 11.4%. Frequency of different STDs observed in descending order was herpes genitalis (28.82%), gonorrhea (8.26%), granuloma inguinal (0.43%) and genital wart (8.9%) [8].

Medicinal plant products have been used as folk remedies for different kinds of ailments including viral diseases. There is a need to search for new compounds for the treatment of viral infections since there is increasing resistance to antiviral drugs. Also, widespread use of chemical drugs has shown resistance especially in immuno-compromised and bone marrow transplant recipients. To circumvent the problem of viral resistance, development of new antiviral products with a different mechanism of action are very much required [9].

Hence, to assess the potential of phytochemicals of *V. phlomoides* L. against target proteins of HSV type I and HSV type II respectively; this research work was carried out.

MATERIALS AND METHODS:

Target Selection: Two targets were selected for this study. HSV-1(PDB ID: 5GIY) was downloaded and used as a template for modeling the structure of HSV 1. The three-dimensional structure was modelled using IntFold server, RaptorX server, SWISS-MODEL program, ModWeb server and Phyre2 [10]. By comparing the results of all the mentioned servers, IntFold server gave the best result. Active sites were predicted using Metapocket server and COACH server [11, 12]. The result of Metapocket server was taken into consideration as it showed the best results. Regions other than active sites in the structure were deleted using SPDBV 4.10 [13].

HSV-2 (PDB ID: 1AT3). Active sites (SER 129 and HIS148) were visualized using Discovery Studio 3.5. HSV-2 contains two chains (chain A and chain B). Active sites were present in chain A hence chain B was deleted. Energy minimization of HSV-1 and HSV-2 was carried out by Chimera

1.11.2, Chiron server, SPDBV 4.10 and validated using Rampage server [13].

Selection of Phytochemicals: Phytochemicals from *Verbascum phlomoides* L. (Apigenin, Apigenin-7-glucoside, Aucubin, Chrysoeriol, Ferulic acid, Kaempferol, Luteolin, Luteolin-7-glucoside, p-coumaric acid, Tamarixetin-7-glucoside, Tamarixetin-7-rutinoside, Verbascosaponin and Verbascoside) shown in **Table 1** were downloaded from PubChem in 2D-SDF format.

ADME/T Properties / Drug Likeness Score: Molecular descriptors and drug likeliness properties of compounds were analyzed using the tool Molsoft server. The most "drug-like "molecules must have log P≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5. Molecules violating more than one of these rules may have problems with oral bioavailability [14].

Seven phytochemicals (ligands) out of thirteen satisfied Lipinski's rule of five. The toxicity of the phytochemicals which satisfied the Lipinski properties was calculated by using the toxicity prediction server-PROTOX [15, 16].

Docking and Visualization: The 7 phytochemicals were docked with the two target protein receptors (HSV-1 and HSV-2). The docking was done using AutoDock Vina 1.5.6. Visualization of docking between target protein receptor and phytochemicals was done using AutoDock Vina 1.5.6 and Discovery Studio 4.2.

RESULTS:

TABLE 1: ADME PROPERTIES OF PHYTOCHEMICALS

S. no.	Phytochemical	Mass (<500Da)	HBD (<5)	HBA (<10)	Log P (<5)	Molar refractivity (40-130)
1	Apigenin	270.000000	0	5	1.488870	61.368496
2	Apigenin7 glucoside	452.000000	4	10	4.264099	118.780174
3	Aucubin	380.000000	3	9	-	-
4	Chrysoeriol	300.000000	0	6	1.649660	67.247993
5	Ferulic acid	193.000000	0	4	0.922120	43.400997
6	Kaempferol	286.000000	1	6	1.189070	62.247295
7	Luteolin	286.000000	0	6	1.558560	62.666996
8	Luteolin7glucoside	466.000000	5	11	3.906789	118.156975
9	p-coumaric acid	163.000000	0	3	0.761330	37.521500
10	Tamarixetin7glucoside	498.000000	6	12	3.944089	125.730774
11	Tamarixetin7rutinoside	668.000000	3	16	-	-
12	Verbascosaponin	1174.000000	0	0	0.00000	0.00000
13	Verbascoside	1174.000000	0	0	0.00000	0.00000

TABLE 2: TOXICITY PREDICTION OF PHYTOCHEMICALS

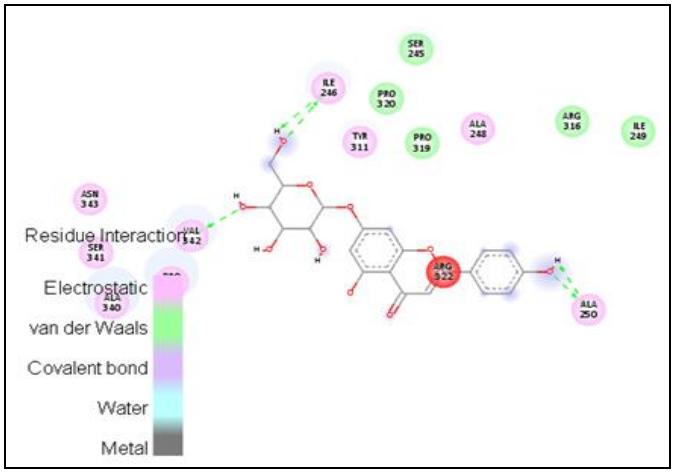
S. no.	Phytochemical	LD_{50} value mg/kg body weight	Toxicity class	Toxic fragment formation	Remark
1	Apigenin	2500	5	None	Non-toxic
2	Apigenin7 glucoside	5000	5	None	Non-toxic
3	Chrysoeriol	4000	5	None	Non-toxic
4	Ferulic acid	1772	4	None	Non-toxic
5	Kaempferol	3919	5	None	Non-toxic
6	Luteolin	3919	5	None	Non-toxic
7	p-coumaric acid	2850	5	None	Non-toxic

- Class I: fatal if swallowed ($LD_{50} \leq 5$ mg/kg)
- Class II: fatal if swallowed ($5 < LD_{50} \leq 50$ mg/kg)
- Class III: toxic if swallowed ($50 < LD_{50} \leq 300$ mg/kg)
- Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$ mg/kg)
- Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$ mg/kg)
- Class VI: non-toxic ($LD_{50} > 5000$ mg/kg)

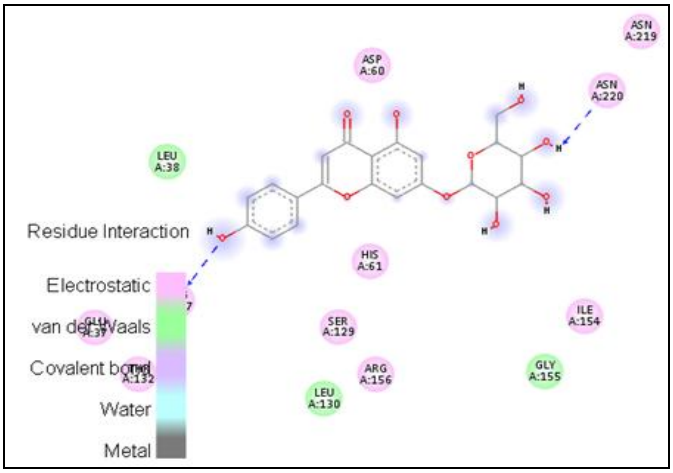
DISCUSSION: Seven phytochemicals (ligands) out of thirteen satisfied Lipinski's rule of 5. The toxicity of the phytochemicals which satisfies Lipinski properties were calculated by using the toxicity prediction server- PROTOX. Compounds with higher lipophilicity were better absorbed from the intestine. Low molecular weight drug molecules (<500) are easily transported, diffuse and absorbed as compared to heavy molecules. Absorption and bile elimination rate both are also molecular weight (MW) dependent. Lower MW gives better absorption and less bile excretion. Molecular weight is an important aspect in therapeutic drug action; if it increases beyond a certain limit, the bulkiness of the compounds also increases correspondingly, which in turn affects the drug action [17].

All the phytochemicals were showing a range of LD_{50} value from 1500-5000 mg/kg and reported to be not generating any toxic fragments.

To investigate the binding affinity of the selected phytochemicals, molecular docking studies were performed. Apigenin7glucoside showed best docking score with both the target proteins- HSV 1 and HSV 2.



2-D INTERACTION OF HSV-1 AND APIGENIN7GLUCOSIDE



2-D INTERACTION OF HSV-2 AND APIGENIN7GLUCOSIDE

The binding affinity of Apigenin7glucoside was seen to be highest, it bonded efficiently to the protein- envelop G of HSV 1 and could aid in preventing the spread of virus in the patient's body since gE is required for the cell-to-cell spread of the virus, is essential for the anterograde spread of the infection throughout the host nervous system and is also involved in the sorting and transport of viral structural components toward axon tips. Serine protease (HSV 2) plays an essential role in virion assembly within the nucleus and hence was targeted. Apigenin7glucoside showed the highest binding affinity to the HSV 2 serine protease target protein. Hence, after further tests and analysis, the selected phytochemicals could be used in formulating drugs against both the types of herpes simplex virus which are currently the major cause for herpes.

CONCLUSION: There is a need for new remedies against herpes simplex virus (HSV) which is a widespread pathogen. Even though the immune system of the host and available antivirals can inhibit the virus, there are situations where the viral disease takes over. In these cases, neither the immune system nor the drugs are capable of preventing damage caused by the infection. In this research work, we present the construction and validation of the HSV-1 structure and a molecular docking study between this structure along with HSV-2 and seven phytochemicals which have cleared ADMET/T filters. Lower binding energies, Drug likeness score, ADME and toxicity of Apigenin-7 glucoside and Luteolin established that it may have drug-like properties and could be a potential hit in the search for new drugs against HSV-1 and HSV-2. However, this present work is only a step forward towards understanding the mechanistic insights of a potent inhibitor for herpes viral infection targeting envelop glycoprotein E of HSV- 1 and the enzyme serine protease of HSV-2, further *in-vitro* and *in-vivo* validations are required after QSAR and molecular dynamic simulation.

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