European Journal of Integrative Medicine 28 (2019) 98–108

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim

Research paper

Antiviral study on *Punica granatum* L., *Momordica charantia* L., *Andrographis paniculata* Nees, and *Melia azedarach* L., to Human Herpes Virus-3

Divyadarshini Angamuthu[a], Indu Purushothaman[a], Sangeetha Kothandan[a], Rajarajan Swaminathan[b,*]

[a] P.G. & Research Department of Microbiology & Biotechnology, Presidency College (Aut), Chennai 05, India
[b] Centre for Designing, Discovery & Development of Drugs, SRM University, Sonepat, Haryana, India

ARTICLE INFO

Keywords:
Human Herpes Virus-3
Varicella Zoster Virus
Antiviral activity
Punica granatum L.
HEp-2

ABSTRACT

Introduction: Human Herpes Virus-3 (Varicella Zoster Virus) causes Chickenpox in childhood and reactivates after decades of being latent to cause Herpes Zoster in adults. The aim of this study was to evaluate the in vitro antiviral potency on the leaves of *Punica granatum* L., *Momordica charantia* L., *Andrographis paniculata* Nees. and *Melia azedarach* L., against the Human Herpes Virus-3 isolated from Chickenpox and Zoster in comparison with acyclovir.

Methods: Aqueous, ethanolic and aqueous ethanolic extracts were prepared from the chosen plant leaves by lyophilization process and subjected to in vitro cytotoxicity assay in HEp-2 cells followed by the in vitro antiviral evaluation against the clinical isolates of HHV-3 using post incubation assay. The structure of leaf chemicals were retrieved from protein data bank and *in silico* drug analysis was carried out through discovery studio targeting the protease of HHV-3.The drug likeliness and the ADMETSAR properties of the screened active phytochemicals were calculated.

Results: Aqueous extract from the leaves of *Punica granatum* L., exhibited potential antiviral activity against the HHV-3. The *in silico* docking results found that the phytochemicals of Punica granatum L., interacted on the active site of the HHV-3 protease.

Conclusion: Aqueous extract from the leaves of *Punica granatum* L. was superior in exhibiting its antiviral efficacy to HHV-3 whose in vitro activity was comparable with acyclovir. As the leaf phytochemicals interacted with the HHV-3 protease, the antiviral activity of the *Punica granatum* L., leaves may interfere with the capsid assembly of the HHV-3.

1. Introduction

Human Herpesvirus-3 (HHV-3 or VZV-Varicella Zoster Virus), an enveloped double stranded DNA virus of the family alpha herpes viruses is ubiquitous, highly contagious and causing one of the most common aerosolized viral infection called Chickenpox or Varicella in children. HHV-3 undergoes latency in the dorsal root of the sensory ganglions and reactivates after several decades owing to the decline in cell immunity or stress to cause Herpes Zoster or Shingles predominantly in adults. However Herpes Zoster has become a viral infection of all age groups [1,2]. HHV-3 also causes several other disease conditions such as varicella pneumonitis [3], meningitis [4], encephalitis [5], post herpetic neuraligia (PHN), herpes zoster ophthalmicus and Ramsay Hunt Syndrome [6]. HHV-3 recurrence and its severity is mainly responsible for the morbidity and hospitalizations in HIV children [7,8] and can also cause death in HIV adults due to intravascular disseminated coagulation [9].

Nucleoside analogues such as acyclovir and its derivatives are being used widely in HHV-3 infections. These drugs require viral encoded thymidine kinase (TK) and cellular kinase for its phosphorylation and inactivate the viral DNA polymerase by competing with guanosine nucleosides that eventually results in the inhibition of viral DNA replication. However these antivirals are associated with side effects such as headache, vomiting, neurotoxic psychological effects [10], renal dysfunction [11] and nephropathy [12]. Also there is a possibility of eliminating unabsorbed drug in the urine due to inadequate oral bioavailability.

Furthermore, the antiviral drug resistance to these nucleoside

* Corresponding author at: Centre for Designing, Discovery & Development of Drugs, SRM University, Sonepat, Haryana, India
E-mail addresses: divyadarshiniangamuthu@gmail.com (D. Angamuthu), indu.purushothaman5@gmail.com (I. Purushothaman), sangee.star@gmail.com (S. Kothandan), drsrajarajan@gmail.com (R. Swaminathan).

https://doi.org/10.1016/j.eujim.2019.04.008
Received 10 October 2018; Received in revised form 30 April 2019; Accepted 30 April 2019

analogues is periodically reported in HIV patients and organ transplanted recipients worldwide. In such cases the constant treatment with these antiviral drugs develops acyclovir resistant HHV-3 and consequently results in chronic, slow healing hyperkeratotic lesions despite high dosage acyclovir therapy [13–15]. The pyrophosphate inhibitor Foscarnet (Phosphano Formic Acid (PFA)) is recommended in the case of acyclovir resistant HHV-3 infections. However resistance to foscarnet is also reported in the literature [16,17,18].

Besides all these issues, the FDA has approved two live attenuated individual vaccines for HHV-3 associated infections namely the varivax for Chickenpox and zostavax for Herpes Zoster. These vaccines have reduced the disease incidence but the generated immunity gives protection for one year. Additionally, the live attenuated Oka vaccine strain can cause vaccine associated break through varicella and zoster infections [19,20].

Therefore, there is a need for a potential antiviral drug with equal efficacy or amplified potential than the existing antiviral drug acyclovir. Phytochemistry remains the main source of potential antimicrobials for various infections and its usage has been in practice since the antique civilization. Globally extensive research is ongoing on the development of antimicrobials from phytokingdom. Various medicinal plants and their phytochemical constituents have been suggested as potential antiviral candidates for Human Herpes Virus-3 [21–28]. However the anti HHV-3 potency in the leaves of *Andrographis paniculata* Nees, *Melia azedarach, Momordica charantia* L., and *Punica granatum* L., is yet to be investigated. Hence, this study was aimed to explore the antiviral potency of these plant leaves on the clinical isolates of HHV-3.

2. Materials and methods

2.1. Cells & viruses

Human epithelial diploid cells (HEp -2) were procured from National Centre for Cell Sciences (NCCS), Pune and were successfully propagated in MEM supplemented with FBS, antibiotics (penicillin 100 IU/ml and streptomycin 100 µg/ml) under 5% CO_2 atmosphere at 37 °C. The HHV-3 clinical isolates from Chickenpox and Herpes Zoster lesions were isolated in HEp-2 cells at 33 °C with prior informed consent. The HHV-3 isolates were confirmed by conventional PCR technique targeting the ORF 62 gene. Briefly the viral DNA was isolated and PCR reaction was carried out with the following primer 5′- GACTTCAACC AGAACCCAGAA-3′ and 3′ ATTACAGGCGAGCCCATTAG-5′. The PCR steps were as follows: initial denaturation for 15 min at 95 °C, followed by 45 cycles of denaturation for 20 s at 95 °C, annealing for 20 s at 56 °C and extension for 20 s at 72 °C. The amplified ORF 62 gene product of HHV-3 isolates were separated in 2% agarose gel and visualized using Bio-Rad Gel documentation system.The tissue culture infectious dose fifty ($TCID_{50}$) was estimated by Reed and Muench method [29]. The viral stocks were stored at −80 °C until further use.

2.2. Collection & processing of plant leaves

The leaves of chosen plants were collected from their habitat and were identified and authenticated in the Department of Plant Biology & Plant Biotechnology at Presidency College, Chennai. The voucher specimen of the respective plant parts were also deposited (#2564,#2565,#2566,#2567). The collected leaves were initially washed in sterile double distilled water and shade dried. The dried leaves were finely grounded in a mixer and sieved to obtain fine powder.

2.3. Preparation of lyophilized extracts

The lyophilized form of aqueous, ethanol and aqueous ethanol extracts from the powdered leaves were prepared by adopting the procedure described by Kothandan S. & Swaminathan R 2014 [30]. Briefly 20 g of each plant leaf powder was soaked in 100 ml of sterile double distilled water (aqueous 100%) and kept for overnight at 4 °C. The same procedure was adopted for ethanolic (100%) and aqueous ethanolic extracts in which the ethanol and water were added in the ratio of 1: 1 (V/V). The aqueous, ethanolic and aqueous ethanolic extracts were squeezed and filtered separately using a gauze cloth. The crude extract was then centrifuged at the speed of 3000 RPM and the clarified supernatant was filtered using a membrane filter apparatus with 0.22 µm Millipore filter. The filtered extracts were loaded into sterile flasks and then lyophilized. The physical properties of the lyophilized extracts were recorded and extracts were stored at 4 °C till further usage.

2.4. In Vitro cytotoxicity assay

The *In Vitro* cytotoxicity of the lyophilized extracts in comparison with the standard drug acyclovir was done by adopting the procedure followed by Julia Serkeidjiva' and Stefka Ivancheva [31].

In brief, a 96 well microtitre plate was seeded according to the required number of wells to perform the *In vitro* cytotoxicity assay. Each well received 100 µl of HEp-2 cell suspension and was then replenished with 100 µl of 10% growth medium. The plate was then allowed to reach overnight confluence at 37 °C under 5% CO_2 humidified atmosphere. The stock solution of the extracts and the standard drug acyclovir (SIGMA Aldrich CAS No: 59277-89-3, ≥99% by HPLC) were prepared and each of the extract was serially diluted through double dilution technique. Hence the drug concentrations 500 µg, 250 µg, 125 µg, 62.5 µg, 31.25 µg, 15.625 µg, 7.8 µg and 3.9 µg/ml were achieved. The growth medium from the confluent titre plate was emptied and 100 µl of the respective drug dilutions were added into the corresponding wells. Then 100 µl of 2% maintenance medium was added and incubated at 37 °C under 5% CO_2 atmosphere for 120 h. The experiment was done in triplicate. The treated HEp-2 cells were observed for every 24 h to record the *in vitro* cytotoxic effects such as unusual morphological variations or changes in cellular shape and size if any. The maximum drug concentration at which there was no absolute *in vitro* cellular toxicity was considered as maximum non toxic concentration (MNTC) of the lyophilized extracts and the standard drug acyclovir.

2.5. In vitro antiviral assay

The *in vitro* antiviral assay of the lyophilized extracts against HHV-3 was performed by adopting the procedure suggested by Ludmila Yarmolinsky et al. [24].

The antiviral assay was performed individually for the clinical isolates of HHV-3 isolated from Chickenpox and Herpes Zoster through post incubation assay in triplicates. Initially, a 96 well titer plate was seeded according to the above said procedure and incubated at 37 °C under 5% CO_2 atmosphere in order to attain the confluence growth. The monolayered wells were infected with 100 µl of diluted viral inoculum with the exclusion of cell control wells in which 100 µl of 2% maintenance medium was added. The virus control well was also set up as positive control. The monolayer in wells was allowed for viral adsorption an hour at 33 °C in 5% CO_2 humidified atmosphere. After adsorption, the unadsorbed viral particles were removed and 100 µl of the respective maximum nontoxic concentration of the serially diluted lyophilized extracts and acyclovir were added into the corresponding wells. The entire set up was incubated at 33 °C under 5% CO_2 atmosphere in a humidified condition for 120 h (5 days). The plates were observed under an inverted phase contrast microscope for every 24 h from the first day of the antiviral evaluation till the end of the experiment to check for the presence or absence of virus induced CPE and the results were recorded with appropriate interpretation. The least drug concentration at which there was no observable viral induced CPE comparatively with cell control was estimated as the Minimum Inhibitory Concentration (MIC) of the respective extracts and acyclovir.

The supernatant from the MIC well was collected and checked for the presence of virus infectivity by infecting the fresh HEp-2 monolayer in comparison with cell control and virus control.

2.6. In silico docking study

The protein structure of protease was retrieved from PDB (ID: 1VZV) and docking was carried out by adopting the procedure described by Erickson et al. [32]. The active site of the VZV protease, in specific the catalytic residues Ser(132)-His(63)-His(157) situated in the A chain (region of the core β-barrel) were selected. The active site was predicted using Discovery Studio version 4.0 and the binding site was defined by current selection method that creates a sphere around the centroid of selected binding site points with the radius adjusted by increasing the sphere radius by 1 A°.

The bound ligand and crystallographic water molecules were removed and loop refinement was carried out to screen the violations and determination of disallowed aminoacids in the protein.

Ligand molecules were prepared and energy was minimized using CHARMm force field in Discovery Studio. Molecular docking was performed by flexible docking analysis. The flexible docking algorithm in Discovery studio allows for receptor flexibility during docking of flexible ligands. Receptor side chain conformations were created using Chiflex algorithm. Low energy ligand conformations were selected and docked into the active site of each receptor side chain conformations using Libdock. The obtained poses are clustered and refined. Furthermore the refined docking poses were subjected for the cDocker energy, binding energy estimation followed by interaction analysis.

2.7. ADMET analysis

The ADMET analysis was carried out based on the ADMETSAR software used by Feixiong Cheng et al. [33]. The ADMET properties such as the blood brain barrier (BBB), human intestinal absorption (HIA) and $CaCo_2$ permeability, mutagenecity and carcinogenecity of the phytochemical compounds were checked through ADMETSAR tool at http://Immd.ecust.edu.cn/admetsar2. The smile format of the compounds was uploaded and the parameters were selected for the ADMET prediction.

Drug likeliness properties were also studied for the compounds using the Lipinski's rule of five which is based on the pharmacokinetic filters such as low molecular weight of five hundred or less, an octanol water partition coefficient log p value of less than five, five or lesser hydrogen bond donor sites, and ten or lesser hydrogen bond acceptor sites (N and O atoms).

3. Results

3.1. In vitro isolation of HHV-3

The inoculated flasks produced HHV-3 CPE consisting of alteration in the cellular morphology, followed by degeneration and disintegration of cells proceeded by dislodging of rounded cells from the flask at the end of 120 h. The CPE produced by the clinical isolates of HHV-3 after 120 h in comparison with control cells was shown in Fig. 1a, b & c.

The ORF 62 gene amplified through Polymerase Chain Reaction has produced a PCR product size of ˜100 bp. The agarose gel image containing ORF 62 gene was shown in Fig. 2.

3.2. Physical properties of the extracts

The physical properties of the extracts such as texture, appearance, color, solubility, pH were recorded. The details of the respective extracts were given in Table 1.

3.3. In vitro cytotoxicity assay

The MNTC of the aqueous and aqueous ethanolic extracts prepared from the leaves of *Punica granatum* L., and *Momordica charantia* L., was 250 µg/ml whereas 125 µg/ml was estimated as MNTC for the ethanolic extracts. The MNTC of the aqueous extract from the leaves of *Andrographis paniculata* Nees. was 62.5 µg/ml whereas the MNTC of the ethanolic and aqueous ethanolic extracts was 31.25 µg/ml and 62.5 µg/ml. The aqueous and aqueous ethanolic extracts from the leaves of *Melia azedarach* L., exhibited MNTC at 62.5 µg/ml and the ethanolic extract was non toxic at from125 µg/ml. The standard drug acyclovir was non toxic at from 500 µg/ml. The MNTC of the extracts were tabulated in Table 2.

3.4. In vitro antiviral assay

The antiviral assay was evaluated individually against the HHV-3 clinical isolates from Chickenpox and Herpes Zoster. The Maximum Non Toxic Concentration (MNTC) of the extracts and acyclovir were considered as the initial drug concentration for the antiviral assay.

Among leaf extracts of four plants evaluated, the aqueous extract of *Punica granatum* L., exhibited a predominant antiviral activity to the clinical isolates of HHV-3 from Chickenpox and Herpes Zoster at its MIC 15.625 µg/ml and 31.25 µg/ml respectively. The ethanolic extract of *Punica granatum* L., inhibited both the HHV-3 isolates at 62.5 µg/ml while its aqueous ethanolic extract inhibited the HHV-3 isolates at 125 µg/ml.

The extracts of *Momordica charantia* L., was effective to HHV-3 isolates at 250 µg/ml in aqueous, 125 µg/ml in ethanolic and was not effective in aqueous ethanolic extracts.

The aqueous extract of *Andrographis paniculata* did not show any significant antiviral activity however the ethanolic extract of this plant inhibited the HHV-3 isolates at its MNTC (i.e.) 31.25 µg/ml and the aqueous ethanolic extract at 62.5 µg/ml.

The ethanolic extract of *Melia azedarach* exhibited antiviral activity at 62.5 µg/ml to the HHV-3 isolated from Chickenpox alone whereas its aqueous and aqueous ethanolic extracts failed to show anti HHV-3 activity.

The standard drug acyclovir inhibited the HHV-3 isolates at 15.625 µg/ml and 31.25 µg/ml.

The detailed anti HHV-3 profile of all the plants leaf extracts studied and acyclovir were tabulated in Table 2. The MIC data was represented in mean ± standard deviation (SD) values and the corresponding figures were illustrated in Figs. 3 and 4.

3.5. Estimation of virucidal activity

With the observed antiviral results, the supernatant collected from the MIC well of the aqueous extract of *Punica granatum* L., and acyclovir were checked for the presence of virus infectivity in HEp 2 cells along with the control set up. Acyclovir treated cells exhibited maximum viral inhibition whereas the infected HEp-2 cells treated with aqueous leaf extract of *Punica granatum* L., have shown considerable viral inhibition at the end of the incubation period.

3.6. In silico docking study

3.6.1. Interaction profile of Punica granatum leaf compounds with 1 VZV

Nine phytochemical leaf compounds of *Punica granatum* L., were retrieved from PDB and docked with the target protein HHV-3 protease (1VZV). Though all the chosen leaf compounds interacted with the target protein, three compounds were scrutinized based on their interaction energy or the binding energy namely apigenin with the highest energy value of −318.299 kcal/mol, followed by isoquercetin with the binding energy value of −220.007 kcal/mol and luteolin with the binding energy value of – 219.607 kcal/mol. The interaction

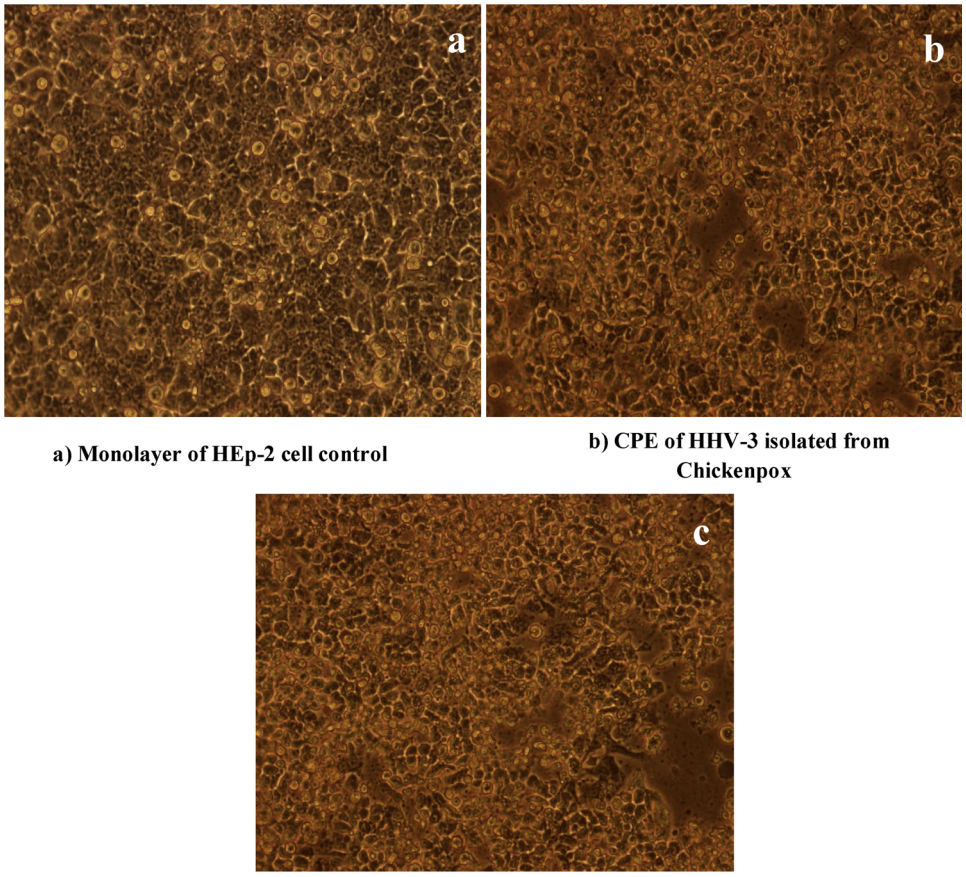

Fig. 1. Cytopathic effect (CPE) of HHV-3 isolated from Chickenpox & Zoster in HEp-2 cell line in comparison with cell control after 120 h (Magnification:40×).

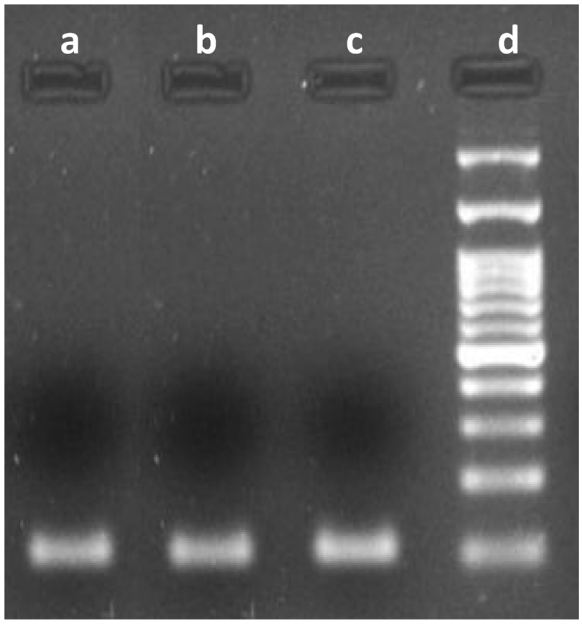

Fig. 2. GEL IMAGE OF ORF 62 GENE OF HHV-3 ISOLATED FROM CHICKENPOX & ZOSTER AMPLIFIED BY PCR.
2% Agarose gel electrophoresis image with the amplified HHV-3 ORF 62 gene of size ˜100 bp isolated from Chickenpox and Herpes Zoster. a) Positive Control; b) amplified ORF 62 gene of HHV-3 isolated from Chickenpox; c) amplified ORF 62 gene of HHV-3 isolated from Zoster and d) 100 base pair ladder.

binding energy of other compounds ranged from −174 to −112 kcal/mol. The details of the top six lead phytochemicals were presented in Table 3. The interaction profile and the binding energy of the lead phytochemicals using lib dock score were given in Table 4.

3.6.2. *Interaction profile of compound Apigenin with 1VZV*

The compound Apigenin exhibited an interaction binding energy value of −318.299 kcal/mol and showed a libDock score of 40.046. It was observed that the compound successfully formed 5 hydrogen bond interactions and it was interesting to note that all the hydrogen bond was exhibited by the oxygen group of Apigenin moiety except for one hydrogen was formed with hydroxyl group with the active site aminoacids GLY146, LEU212, ARG148 and ARG147.The bond length was also significantly high ranged from 1.94 to 3.05 Å (Fig. 5a). Since all the hydrogen bonds were found to be significant and few pi pi interactions were also observed.

3.6.3. *Interaction profile of compound isoquercetin with 1VZV*

The compound isoquercetin exhibited the binding energy value of -220.007 kcal/mol with libDock score of 44.65. Despite of low binding energy, it was surprising to note that the compound formed 15 hydrogen bond interactions with the amino acids LEU 121, ASN 30 and GLU 28 forming hydroxyl moiety with the active site and SER123, ARG 148, ARG 147, forming oxygen interaction moiety. One pi pi interaction was also observed with the ARG 147 of the protein. The bond length was found to be 1.89–2.83 Å (Fig. 5b).

3.6.4. *Interaction profile of compound luteolin with 1VZV*

The compound luteolin exhibited the binding energy value of -219.607 kcal/mol with a libDock score of −37.759. Similar to the

Table 1
Physical properties of the extract.

Name of the plant	Extract type	Appearance	Solubility	pH	Total yield
***Punica granatum* L.**	Aqueous	Chocolate **brown** coarse crystals	Water	7.0	3.2g
	Ethanol	Greenish **brown** coarse crystals	Ethanol	7.5	2.4 g
	Aqueous Ethanol	Pale **brown** coarse powder	Water	7.0	2.7 g
***Momordica charantia* L.**	Aqueous	Dark **brown** coarse powder	Water	7.0	4.3 g
	Ethanol	Dark green and pasty	Ethanol	7.0	3.1 g
	Aqueous Ethanol	Dark **brown** coarse crystals	Water	6.5	4 g
***Andrographis paniculata* Nees.**	Aqueous	Dark green glassy crystals	Water	7.0	10.6 g
	Ethanol	Dark green coarse powder	DMSO	7.5	8.7g
	Aqueous Ethanol	Chocolate **brown** coarse powder	DMSO	7.5	9.3 g
***Melia azederach* L.**	Aqueous	Dark **brown** coarse crystals	Water	7.0	3.9 g
	Ethanol	Dark green & pasty	Ethanol	6.5	2.8 g
	Aqueous Ethanol	Dark **brown** and pasty	DMSO	6.5	2.5g

Table 2
Inhibitory activity of the chosen extracts and acyclovir against the HHV-3 isolated from Chickenpox and Zoster.

Plant	Type of extract	MNTC of the extracts (µg/ml)	HHV-3 ISOLATED FROM CHICKENPOX MIC(µg/ml)	HHV-3 ISOLATED FROM ZOSTER MIC(µg/ml)	SELECTIVITY INDEX	
***Punica granatum* L.,**	Aqueous	250 ± 65.3	15.625 ± 7.3	31.25 ± 7.3	16	8
	Ethanol	125 ± 16.7	62.5 ± 14.65	62.5 ± 14.62	2	2
	Aqueous Ethanol	250 ± 52.7	125 ± 58.6	125 ± 58.5	2	2
***Momordica charantia* L.,**	Aqueous	250 ± 63.2	250 ± 58.6	250 ± 58.6	1	1
	Ethanol	125 ± 8.6	62.5 ± 14.6	62.5 ± 14.6	2	2
	Aqueous Ethanol	250 ± 54.3	–	–	–	–
***Andrographis paniculata* Nees.**	Aqueous	62.5 ± 14.2	–	–	–	–
	Ethanol	31.25 ± 20.5	31.25 ± 7.1	31.25 ± 6.9	1	1
	Aqueous Ethanol	62.5 ± 11.2	62.5 ± 14.6	62.5 ± 14.6	1	1
***Melia azedarach* L.,**	Aqueous	62.5 ± 13.8	–	–	–	–
	Ethanol	125 ± 12.8	62.5 ± 14.6	–	2	–
	Aqueous Ethanol	62.5 ± 7.3	–	–	–	–
Acyclovir		500 ± 58.5	15.625 ± 7.3	31.25 ± 7.3	32	16

NOTE: MNTC of the extracts and MIC values expressed in mean ± SD.

isoquercetin low binding energy was exhibited by the compound luteolin and had formed 3 hydrogen bond interactions with the aminoacids. ARG 148 involved in the formation of hydroxyl moiety with the compound luteolin and ARG 147 and LYS 124 formed oxygen based moiety. In this compound, two pi pi interaction were observed with ARG 148 and LYS 124. The bond length of hydrogen bond interaction ranged between 2.37 and 2.58 Å (Fig. 5c).

3.6.5. Interaction profile of compound Granatin A with 1VZV

The compound granatin A exhibited the binding energy value of −174.581 kcal/mol with a libDock score of −60.937 and formed 6 hydrogen bond interactions. The aminoacids SER 120 and SER 123 were involved in the formation of carbon and nitrogen based hydroxyl moiety with the compound granatin A whereas the aminoacids HIS 52 and SER 123 contributed the formation of oxygen based moiety. There was no trace of pi pi interaction with the active site amino acids. The bond length of hydrogen bond interaction ranged between 1.81and 2.7 Å (Fig. 5d).

3.6.6. Interaction profile of compound Strictinin with 1VZV

The compound Strictinin exhibited the binding energy value of -113.517 kcal/mol with a libDock score of −36.28 and exhibited 9 hydrogen bond interactions with the active site aminoacids. SER 122, VAL 145, LEU 29, ARG 147 was involved in the formation of carbon and nitrogen based hydroxyl moiety with the Strictinin and SER 123, VAL 145, ASN 209, ASP51 exhibited oxygen based moiety. The compound did not exhibit any pi pi interaction and the bond length ranged between 1.81and 2.84 Å (Fig. 5e).

3.6.7. Interaction profile of compound Corilagin with 1VZV

The compound Corilagin exhibited the binding energy value of −112.24 kcal/mol with a libDock score of −48.215 and exhibited 8 hydrogen bond interactions with the active site aminoacids. SER 122, SER 123, ARG 148 and ARG 125 were involved in the formation of carbon and nitrogen based hydroxyl moiety with the Corilagin and SER 123 and ASN 30 exhibited oxygen based moiety. The compound did not exhibit any pi pi interaction and the hydrogen bonding was observed with the bond length range between 1.81 and 2.74 Å (Fig. 5f).

3.7. ADMETSAR profile

The ADMETSAR profile of the screened phytochemical compounds had shown that the compounds luteolin and isoquercetin satisfied for all except for the blood brain barrier and $CaCO_2$ permeability while the compound apigenin was found to satisfy all parameters. These three compounds were found to be non AMES toxic and non carcinogen as well. The details are given in Table 5.

4. Discussion

Globally the need for the potential and novel antiviral agents is increasing as the diverse viral infections are posing a great threat to the human population. Despite the fact of associated adverse effects with the antiviral drugs, the researchers are thriving to seek out the potential alternate source of antiviral agents. Medicinal plants have been in use for controlling various microbial infections since from the prehistoric times. The promising research findings on various higher and lower

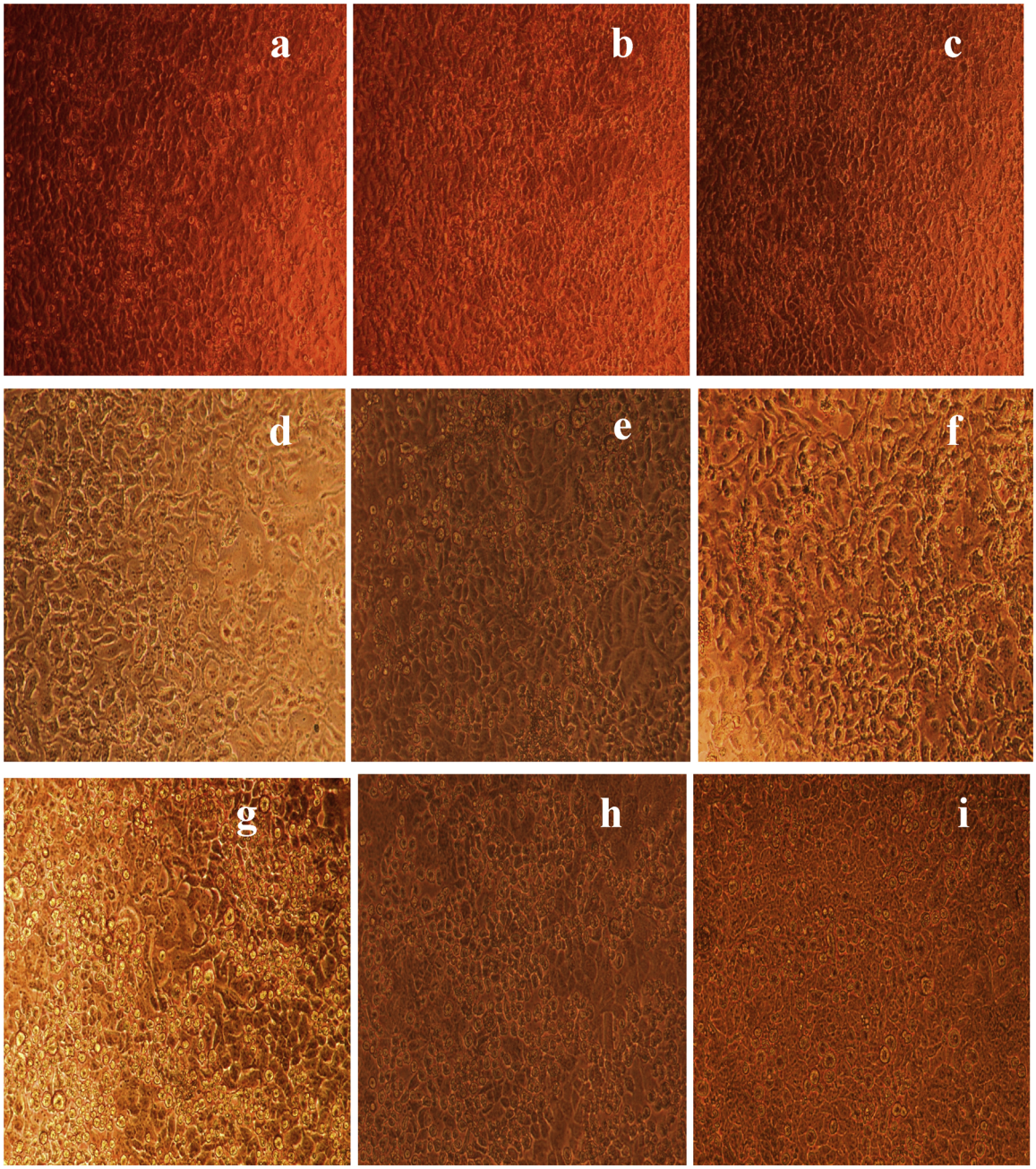

Fig. 3. In vitro antiviral activity of plant extracts to the HHV-3 isolated from Chickenpox (Magnification: 40×).
In vitro anti HHV-3 results (Chickenpox HHV-3) showing MIC for the lyophilized extracts from the leaves of chosen plants: a) Aqueous extract of *Punica granatum* L., at 15.625 µg/ml; b) Ethanolic extract of *Punica granatum* L., at 62.5 µg/ml; c) Aqueous ethanolic extract of *Punica granatum* L., at 125 µg/ml; d) Aqueous extract of *Momordica charantia* L., at 250 µg/ml; e) Ethanolic extract of *Momordica charantia* L., at 62.5 µg/ml; f) Ethanolic extract of *Andrographis paniculata* Nees. at 62.5 µg/ml; & g) Aqueous ethanolic extract of *Andrographis paniculata* Nees. at 62.5 µg/ml; h) ethanolic extract of *Melia azedarach* at 62.5 µg/ml & i) acyclovir at 15.625 µg/ml

plants emphasize the view that the phytokingdom is an important natural source of incredible antimicrobials.

This study highlights the anti HHV-3 potency in the leaves of chosen plants namely the *Punica granatum* L., *Momordica charantia* L., *Andrographis paniculata* Nees and *Melia azedarach* L, Previously the antiviral activity residing in several higher plants namely the *Pongamia pinnata* [22], *Ficus benjamina, Lilium candilum* [23] and Passiflora *edulis* [24] were extensively studied for other members of alpha herpes viruses. However the antiviral studies on Human Herpes Virus-3 were limited with special reference to plants and also the antiviral efficacy residing in the chosen plant leaves was annulled in the literature. Hence with this idealistic approach, the *in vitro* antiviral screening of the above said four plant leaves were performed individually against the clinical isolates of HHV-3 from Chickenpox and Herpes Zoster. Also it is noteworthy to mention that this present work is the first study to report on the anti HHV-3 property of the above said four plants.

While the authors O'Neil et al. 1996 [34], Ozaki, T.K., et al., 1996 [35] have propagated HHV-3 in Human embryonic cells (MRC-5) at 37 °C, we have isolated HHV-3 from the vesicular fluid lesions of the Chickenpox and Herpes Zoster patients at 33 °C in HEp-2 cells, an easily accessible and hardy cells with an unlimited lifespan. In our study the CPE of HHV-3 was visible on the 5th day of inoculation and had reached a higher titre value of MOI of 1. Grose, C and Brunel, P.A. 1978 [36] have isolated the VZV in high titre when incubated at temperature below 36 °C in Human melanoma malignant cells and in our study, HHV-3 was successfully isolated at high titre using HEp-2cells at 33 °C.

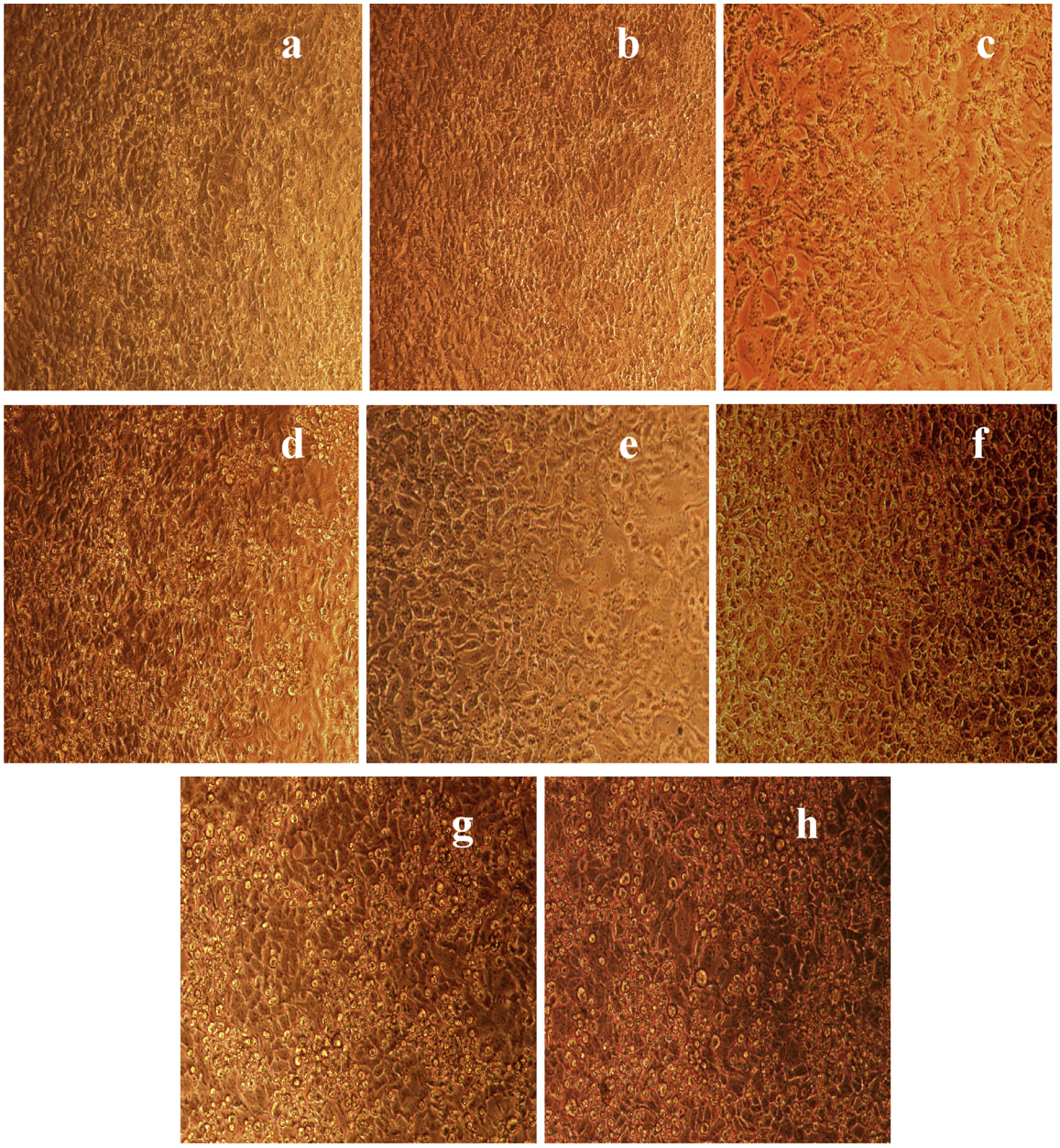

Fig. 4. In vitro antiviral activity of plant extracts to the HHV-3 isolated from Herpes Zoster (Magnification: 40×).
In vitro anti HHV-3 results (Herpes Zoster HHV-3) showing MIC for the lyophilized extracts from the leaves of chosen plants: a) Aqueous extract of *Punica granatum* L., at 31.25 µg/ml; b) Ethanolic extract of *Punica granatum* L., at 62.5 µg/ml; c) Aqueous ethanolic extract of *Punica granatum* L., at 125 µg/ml; d) Aqueous extract of Momordica charantia L., at 250 µg/ml; e) Ethanolic extract of *Momordica charantia* L., at 62.5 µg/ml; f) Ethanolic extract of *Andrographis paniculata* Nees. at 62.5 µg/ml; g) Aqueous ethanolic extract of *Andrographis paniculata* Nees. at 125 µg/ml; h) Acyclovir at 31.25 µg/ml

Table 3
The best six lead molecules and their important properties.

S. No.	Name	LibDock Score	Binding Energy (Kcal/mol)	Total number of Hydrogen Bond
1	Apigenin	40.046	−318.299	5
2	Isoquercetin	44.655	−220.007	15
3	Luteolin	37.759	−219.607	3
4	Granatin A	60.937	−174.581	6
5	Strictinin	36.28	−113.517	9
6	Corilagin	48.215	−112.24	8

Various identification techniques have been used for confirming the HHV-3 isolates namely the monoclonal fluorescent antibody [37] and direct immunoflourescence techniques [38]. In this study, the HHV-3 isolates were confirmed using conventional PCR technique by targeting ORF 62 gene amplification which supports the results of Vladimir N. Loparev et al., 2000 [39] in which the VZV isolates were identified by amplification of ORF 62 gene using PCR technique.

Among the four chosen plant leaves, the lyophilized *Punica granatum* L., leaf extracts exhibited limited *in vitro* cytotoxicity to the HEp-2 cells as it was evidenced from their Maximum Non Toxic Concentration (MNTC). The MNTC of the extracts from *Momordica charantia* L., *Andrographis paniculata* Nees. and *Melia azedarach* L., were slightly higher than the MNTC observed with the *Punica granatum* L., leaf extracts. This is the first study to report on the *in vitro* cytotoxicity of the aqueous, ethanolic and aqueous ethanolic lyophilized extracts from the leaves of the chosen plant leaves in HEp-2 cells.

The maximum non toxic concentration (MNTC) of acyclovir is

Table 4
Interaction profile and binding energy of lead molecules using libDock algorithm.

Compounds	Binding energy	Hydrogen bond interaction	Distance in Å
Apigenin	**-318.299**	GLY146 C—H…O	2.58
		GLY146 C—H…O	2.83
		O—H…O LEU121	2.31
		ARG148 C—H…O	3.05
		ARG147 N—H…O	1.94
Isoquercetin	**−220.007**	C—H.O LYS 124	2.48
		SER 120 O—H.O	2.62
		ARG 147 C—H.O	2.8
		O—H.O SER123	2.9
		O—H.O ASN 30	2.6
		O—H.O ASN 30	2.45
		O—H.O GLU 28	2.02
		C—H.O GLU 28	2.47
		LEU 29 C—H.O	2.83
		ARG148 N—H…O	2.57
		ARG148 N—H…O	2.62
		SER123 C—H.O	2.9
		SER123 C—H.O	2.69
		ARG148 N—H…O	1.89
		O—H.O LEU 121	1.96
Luteolin	**−219.607**	LEU 29C—H.O	2.37
		ARG 148 N—H…O	2.42
		THR 138 O—H.O	2.58
		SER 52 N—H.O	2.62
		O—H.N HIS 139	2.61
		SER 123 N—H.O	2.65
		SER 123 C—H.O	2.7
		ARG 148 N—H..0	2.48
		HIS 52 N—H.O	2.63
		O—H….O SER 123	2.01
		GLY 124 C-H.O	2.28
		C—H.O -LYS124	2.38
Granatin A	**−174.581**	O—H.O SER 120	2.46
		HIS 52 C—H.O	1.81
		SER 123 C—H.O	2.7
		O—H….O SER 123	1.9
		O—H….O SER 123	2.68
		SER 123 C—H.O	2.55
Strictinin	**−113.517**	O—H….O SER 123	2.11
		SER 122 C—H.O	2.47
		O—H.O VAL 145	1.97
		VAL 145 N—H.O	1.81
		O—H.O ASN 209	2.25
		O—H.O ASP 51	2.6
		LEU 29 C—H.O	2.43
		ARG 147 N—H.O	2.58
		SER 122 C—H.O	2.84
Corilagin	**–112.24**	O—H.O ASN 30	2.34
		ARG 148 N—H.O	1.81
		SER 123 C—H.O	2.44
		O—H….O SER 123	1.99
		SER 123 C—H.O	2.31
		SER 122 N—H.O	2.74
		SER 122 C—H.O	2.5
		ARG 125 N—H.O	2.56

500 µg/ml and is higher than the *in vitro* non toxic concentration of acyclovir reported by Rania I Shebl *et* al.2012 which was 250 µg/ml. Hence it is obvious with our *in vitro* cytotoxicity assay result that acyclovir do not possess any *in vitro* cytotoxicity at higher drug concentration.

In this present work, the antiviral activity was evaluated individually against the clinical isolates of HHV-3. *Andrographis paniculata* Nees. exhibited anti HHV-3 activity only in its ethanolic extract at 31.25 µg/ml. However the MIC observed with respect to this plant leaf ethanolic extract was higher than the antiviral concentration achieved with the ethanolic extract of *Andrographis paniculata* by Lin *et* al., reported for Epstein Barr Virus at 25 µg/ml [40]. Among the four chosen plants, this plant had exhibited potential *in vitro* cytotoxicity to the HEp-2 cells. Hence the safety aspects raise its question of taking up for further antiviral studies.

The studies on the antiviral evaluation of *Melia azedarach* L., reports of its promising antiviral activity however the ethanolic extract alone was effective towards the HHV-3 isolate at 62.5 µg/ml in our study. The extracts of *Momordica charantia* L., also had shown considerable activity at 250 µg/ml in aqueous and 125 µg/ml in ethanolic extracts. This MIC was much lesser than the antiviral drug concentration of an anti influenza protein (1.402 mg/ml) reported from the seeds of *Momordica charantia* L., by Pongthanapisith et al., 2013 [41].

To our surprise, we found that the lyophilized aqueous extract from the dried leaves of *Punica granatum* L., exhibited an appreciable and a dominant antiviral activity to both the clinical isolates of HHV-3. This plant leaf aqueous extract was highly effective in preventing the HHV-3 induced cytopathic effect in HEp-2 cells than either the ethanolic or aqueous ethanolic extracts. The aqueous extract inhibited the HHV-3 isolates at its MIC 15.625 µg/ml (HHV-3 isolated from Chickenpox) and at 31.25 µg/ml (HHV-3 isolated from Zoster) respectively whose inhibitory activity was much superior to the anti- HHV-3 activity of the licorice powder extract containing 125 µg of glycyrrhizin observed by Shebl. 2012 [25].

In previous study Mohamad-Tahgi Moradi et al 2015 [42] had evaluated the anti HHV-1 activity of crude ethanolic extract from the fruit rind of *Punica granatum* L., and found that HHV-1 was inhibited in the adsorption stage with an IC50 value of 37.7 ± 6/7 µg/ml in vero cells. Though this study supports the antiviral potency of *Punica granatum* L., our finding is new that has revealed the antiviral activity of the leaves of *Punica granatum* L., to the HHV-3 isolates. Also it is noteworthy to mention that the leaf extracts possibly would have caused an interruption in the replication of VZV as the antiviral assay was done and evaluated post infection till the final day of observation. However the research finding by Ludmila Yarmolinsky et al 2010 [24] found that the aqueous extract of *Cassiflora fragrans* have caused only partial inhibition of HHV-3 post infection in vero cells.

Additionally, the aqueous leaf extract of *Punica granatum* L., had shown a comparable antiviral inhibition with the standard acyclovir whose MIC was 15.625 µg/ml and 31.25 µg/ml for the HHV-3 isolates and hence these antiviral drug concentration were checked for the presence of any endogenous viruses. Through this technique, it was found that the aqueous extract of *Punica* plant had considerably inhibited the viral infectivity of HHV-3 clinical isolates from Chickenpox and Herpes Zoster lesions as there was not any significant virus induced CPE in the infected cells as like that of acyclovir. This is an important research finding in our antiviral study which says that the aqueous extract from the leaves of *Punica granatum* L., would have possibly interacted with the viruses and interfered in the viral life cycle. Also, the selectivity index of the aqueous extract of *Punica granatum* L., was higher than the selectivity index of the extracts prepared from other chosen plants. This data supports the suitability of the leaves of *Punica* as an antiviral drug as the selectivity index suggests that it may not have any interaction with the host cells while exhibiting the antiviral activity.

Besides these the ethanol and the aqueous ethanol extracts of *Punica granatum* L., also inhibited the HHV-3 isolates at higher drug concentrations. Also the *Punica* plant did not exhibited much *in vitro* cytotoxicity at from higher drug concentrations seen for other chosen plants. Hence with these observations, we emphasize the fact that the antiviral medicinal properties to HHV-3 are found in the leaves of *Punica granatum* L., plant.

Furthermore, we attempted to screen the active phytochemicals which were present in the leaves of *Punica granatum* L., through *in silico* study. Molecular docking is a method which predicts the preferred orientation of one molecule to that of the other when bound to each other in forming a stable complex and it plays an important role in rational drug design [43,44].

This inspired us to perform a docking study for the phytocompounds from the leaves of *Punica granatum* L., with the target protein, HHV-3

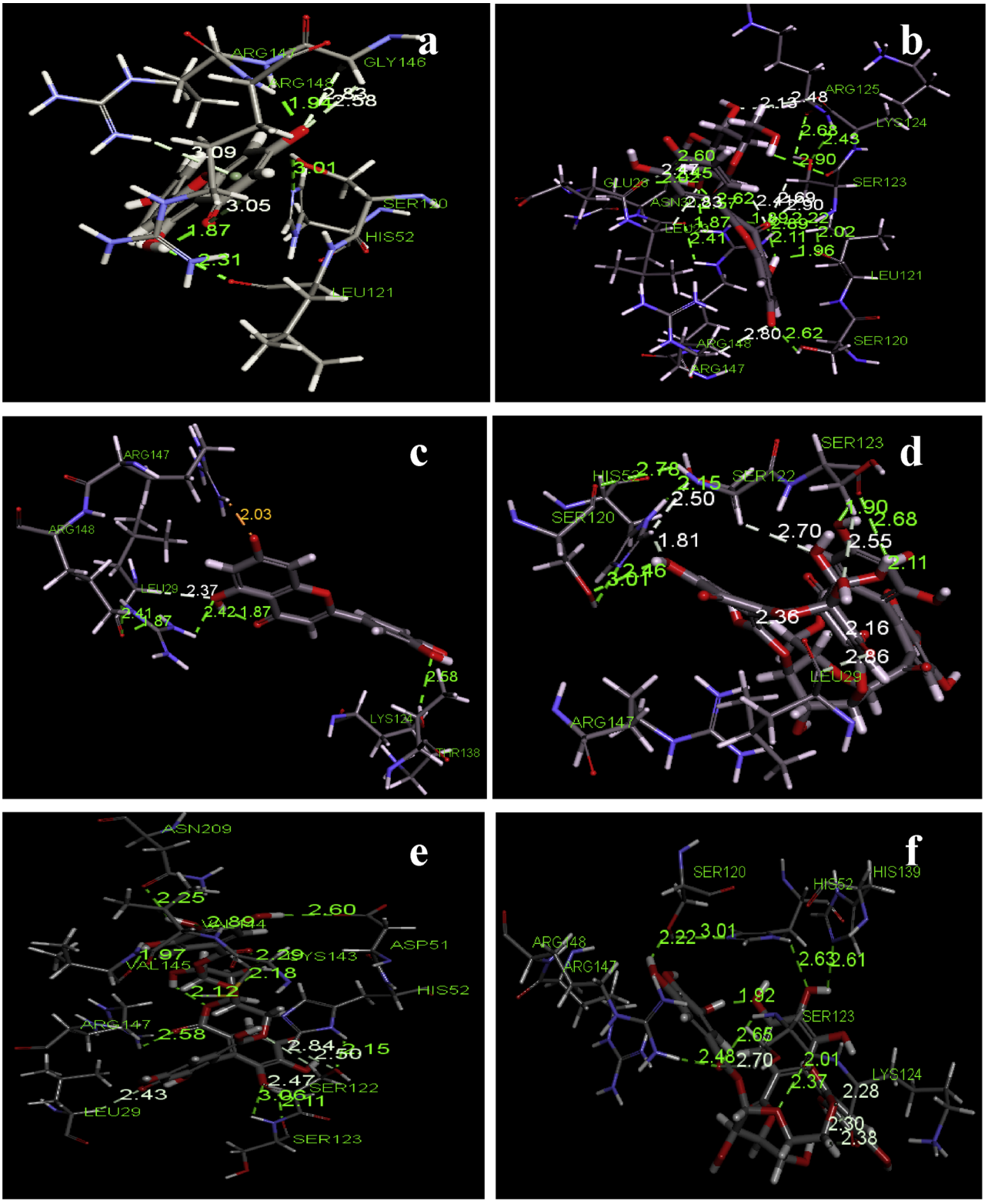

Fig. 5. The interaction profile of the lead molecules with the active site residues of HHV-3 protease.
Docked confirmation of the phytochemicals with the HHV-3 protease. a) Apigenin; b) isoquercetin; c) Luteolin; d) Granatin A; e) Strictinin & f) Corilagin. The green colour indicates the amino acids involved in the hydrogen bond formation, orange colour show the amino acids that forms pi pi interaction, thick wireframe structure depicts the ligand.

protease, a major protein involved in the capsid assembly and DNA packaging of HHV-3 during the late event of viral multiplication. Docking results showed that Apigenin had the highest binding affinity against the protease of HHV-3 with a binding energy value of -318.299 Kcal/mol forming hydrogen bond with GLY146, LEU212, ARG148 and ARG147 and the bond length falls between 1.9 to 3.4 Å followed by isoquercetin with a binding energy value of -220.007 Kcal/mol forming hydrogen bond with LYS 124, SER 120, ARG 147, SER123, ASN 30,

Table 5
ADMETSAR RESULTS FOR THE PHYTOCHEMICALS.

ANALYSIS	APIGENIN	ISOQUERCETIN	LUTEOLIN
Blood Brain Barrier	BBB +	BBB-	BBB-
Human Intestinal Absorption	HIA +	HIA +	HIA +
$CaCO_2$ permeability	$CaCO_2$ +	$CaCO_2$ -	$CaCO_2$ -
TOXICITY			
AMES toxicity	Non AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogenicity	Non-carcinogen	Non-carcinogen	Non-carcinogen

GLU 28, LEU 29, ARG148, SER123, LEU 121 and exhibited a bond length between 1.8 and 2.8 Å and luteolin showed a binding energy value of -219.607 Kcal/mol forming hydrogen bond with HHV-3 protease which was more effective than the previous study reported by Angamuthu et al. [45] who reported that Erysenegalensein E had the highest binding affinity value of −114.4 using iGEMDOCK and it formed hydrogen bond with ILE 63, ILE 64 with the HHV-3 protease. Hence all the above said active phytocompounds of *Punica granatum* L., interacted with the catalytic site of the HHV-3 protease and were thought to be involved in the inhibition of the capsid assembly of HHV-3. Though the phytocompounds granatin A, Strictinin and Corilagin had exhibited binding affinity against the protease of HHV-3, their binding energy was comparatively lower when compared to the screened phytocompounds. Hence Apigenin, isoquercetin and luteolin were taken up for further studies.

The Lipinski`s rules (RO5) states that drug molecules should exhibit good absorption or permeation when they have an octanol-water partition coefficient (miLog P) < 5.Thus, the drug likeliness property analysis revealed that the screened compounds has a lower log P value which corresponds to better bioavailability of the drugs. The ADMETSAR results found that the phytochemicals apigenin, isoquercetin and luteolin were found to be non AMES toxic and non carcinogen as well.

Hence with the results observed, this study illustrates the anti HHV-3 potency of the leaves of *Punica granatum* L., a plant which is commonly seen in the native regions of Indian subcontinent. This study has to be further explored for the isolation of active phytochemicals and evaluating its *in vitro* anti-viral efficacy against the drug resistant HHV-3 strains which may lay a concrete towards the goal of achieving a potent antiviral drug against the HHV-3 infections. Pongracic. 2002 [46] had performed the docking study targeting the viral enzyme thymidine kinase and reported that series of novel 9-(2hydroxypropyl) purine derivatives believed to have better interaction energy when compared to other compounds.

Similarly Shakya et al. 2015 [47] had performed a docking study targeting the ORF 61 of HHV-3 and predicted that the molecule 4AYC_CPQ_A_1489 had a binding energy of value – 12.5 using Auto Dock. From all these studies it can be concluded that though there are various protein targets for HHV-3, our study is a new one with an objective of suggesting the leaves of *Punica granatum* L., as an effective antiviral against the clinical isolates of HHV-3 and the screened phytochemicals targeting the HHV-3 protease. Though we have highlighted the antiviral potency in the leaves of *Punica granatum* L., against the HHV-3, the mode of action of the active phytochemical was predicted by a preliminary *in silico* analysis and was not substantiated further through *in vitro* results, which is the limitation of this antiviral study.

5. Conclusion

Through this antiviral research work, we conclude that the leaves of *Punica granatum* L., has potential antiviral mechanism against the HHV-3. Hence the leaves of *Punica granatum* L., has to be further explored for the isolation of active antiviral phytochemicals. We also conclude that these phytochemicals can further be investigated for the presence of *in vitro* antiviral activity against the HHV-3 followed by the *in vivo* safety studies and *in vivo* antiviral studies. Also we found that the leaf phytochemicals of *Punica* interact with the catalytic site of the HHV-3 protease and hence these phytochemicals could be developed as an alternate drug target for the treatment of HHV-3 infections.

Authors

All research done by the authors.

Financial Support

No.

Conflict of interest

None.

Acknowledgements

The authors are extremely thankful to the Department of Science and Technology, Govt of India for providing an excellent laboratory facility to the PG & Research Dept. of Microbiology & Biotechnology, Presidency College, Chennai-05 where the research activity was carried out. The authors also acknowledge RKK Medical Centre, Chennai for their rendered help in the collection of clinical samples during the study.

References

[1] R.C. Sharma, Clinical profile of cutaneous infections and infestations in the pediatric age group, Indian J. Dermatol. 44 (04) (1999) 174–178.
[2] A.K. Dubey, T.J. Jaisankar, D.M. Thappa, Clinical and morphological characteristics of herpes zoster in south India, Indian J. Dermatol. 50 (4) (2005) 203–207.
[3] M. Sans, J.M. Gatell, M. Rafel, J. Mallolas, E. Soriano, Varicella pneumonia in adults infected by HIV-1. Presentation of 2 cases, Enferm. Infecc. Microbiol. Clin. 12 (1) (1994) 26–30.
[4] B.A. Cunha, H. Warren-Favorito, N. Mickail, Unusually severe varicella zoster (VZV) virus viral (aseptic) meningitis in an unimmunized, immunocompetent host with chickenpox, Heart Lung: J. Acute Crit. Care 40 (4) (2011) 349–351.
[5] T. De Broucker, A. Mailles, S. Chabrier, P. Morand, J.P. Stahl, Acute varicella zoster encephalitis without evidence of primary vasculopathy in a case-series of 20 patients, Clin. Microbiol. Infect. 18 (8) (2012) 808–819.
[6] K.K. Adour, Otological complications of herpes zoster, Ann. Neurol. 35 (1) (1994) 62–64.
[7] E. Jura, E.G. Chadwick, S.H. Josephs, S.P. Steinberg, R. Yogev, A.A. Gershon, et al., Varicella-zoster virus infections in children infected with human immunodeficiency virus, Pediatr. Infect. Dis. J. 8 (9) (1989) 586–590.
[8] L. Von Seidlein, S.G. Gillette, Y. Bryson, T. Frederick, L. Mascola, J. Church, et al., Frequent recurrence and persistence of varicella-zoster virus infections in children infected with human immunodeficiency virus type 1, J. Pediatr. 128 (1) (1996) 52–57.
[9] C. Perronne, M. Lazanas, C. Leport, F. Simon, D. Salmon, A. Dallot, et al., Varicella in patients infected with the human immunodeficiency virus, Arch. Dermatol. 126 (8) (1990) 1033–1036.
[10] D. Niibori, M. Fujisawa, M. Matsuzaki, Severe central nervous system symptoms following oral administration of acyclovir in a patient with chronic renal failure: a case report. Hinyokika kiyo, Acta Urol. Japonica 39 (5) (1993) 459–461.
[11] P.K. Sodhi, S.K. Ratan, A case of chronic renal dysfunction following treatment with oral acyclovir, Scand. J. Infect. Dis. 35 (10) (2003) 770–772.
[12] R. Fleischer, M. Johnson, Acyclovir nephrotoxicity: a case report highlighting the importance of prevention, detection, and treatment of acyclovir-induced nephropathy, Case Rep. Med. (2010) 1–3.
[13] S. Pahwa, K. Biron, W. Lim, P. Swenson, M.H. Kaplan, N. Sadick, et al., Continuous varicella-zoster infection associated with acyclovir resistance in a child with AIDS, JAMA 260 (19) (1988) 2879–2882.
[14] M.A. Jacobson, T.G. Berger, S. Fikrig, P. Becherer, J.W. Moohr, S.C. Stanat, et al., Acyclovir-resistant varicella zoster virus infection after chronic oral acyclovir therapy in patients with the acquired immunodeficiency syndrome (AIDS), Ann. Intern. Med. 112 (3) (1990) 187–191.
[15] A.A. Brink, M. van Gelder, P.F. Wolffs, C.A. Bruggeman, I.H. van Loo, Compartmentalization of acyclovir-resistant varicella zoster virus: implications for sampling in molecular diagnostics, Clin. Infect. Dis. 52 (8) (2011) 982–987.
[16] S. Safrin, T.G. Berger, I. Gilson, P.R. Wolfe, C.B. Wofsy, J. Mills, et al., Foscarnet therapy in five patients with AIDS and acyclovir-resistant varicella-zoster virus

infection, Ann. Intern. Med. 115 (1) (1991) 19–21.
[17] B. Visse, B. Dumont, J.M. Huraux, A.M. Fillet, Single amino acid change in DNA polymerase is associated with foscarnet resistance in a varicella-zoster virus strain recovered from a patient with AIDS, J. Infect. Dis. 178 (1) (1998) 55–57.
[18] B. Visse, J.M. Huraux, A.M. Fillet, Point mutations in the varicella-zoster virus DNA polymerase gene confers resistance to foscarnet and slow growth phenotype, J. Med. Virol. 59 (1) (1999) 84–90.
[19] P. La Russa, S. Steinberg, F. Meurice, A. Gershon, Transmission of vaccine strain varicella-zoster virus from a healthy adult with vaccine-associated rash to susceptible household contacts, J. Infect. Dis. 176 (4) (1997) 1072–1075.
[20] I. Hardy, A.A. Gershon, S.P. Steinberg, P. LaRussa, The incidence of zoster after immunization with live attenuated varicella vaccine: a study in children with leukemia, N. Engl. J. Med. 325 (22) (1991) 1545–1550.
[21] M.R. Roner, J. Sprayberry, M. Spinks, S. Dhanji, Antiviral activity obtained from aqueous extracts of the Chilean soapbark tree (Quillaja saponaria Molina), J. Gen. Virol. 88 (1) (2007) 275–285.
[22] M. Elanchezhian, S. Rajarajan, P. Rajendran, S. Subramanian, S.P. Thyagarajan, Antiviral properties of the seed extract of an Indian medicinal plant, Pongamia pinnata, Linn. Against herpes simplex viruses: in vitro studies on vero cells, J. Med. Microbiol. 38 (1993) 262–264.
[23] L. Yarmolinsky, M. Zaccai, S. Ben-Shabat, D. Mills, M. Huleihel, Antiviral activity of ethanol extracts of *Ficus benjamina* and Lilium *candilum* in vitro, N. Biotechnol. 26 (6) (2009) 307–313.
[24] L. Yarmolinsky, M. Zaccai, S. Ben-Shabat, M. Huleihel, Anti-herpetic activity of Callissia fragrans and Simmondsia chinensis leaf extracts in vitro, Open Virol. J. 4 (2010) 57–63 [PMID:20700398].
[25] Rania I. Shebl, Magdy A. Amin, Amal Emad-Eldin, Saad M. Bin Dajem, Amal S. Mostafa, Essam H. Ibrahim, Aly F. Mohamed, Antiviral activity of liquorice powder extract against Varicella Zoster Virus isolated from Egyptian patients, Chang Gung Med. J. 35 (3) (2012) 231–239 [PMID 22735054].
[26] Azhar Jabareen, Mahmoud Huleihil, Mahmoud Huleihel, Effect of extracts of *Passiflora edulis* leaves on Herpes viruses infection, J. Virol. Antivirus Res. 2 (2) (2013) 1–6.
[27] John J. Docherty, Thomas J. Sweet, Erin Bailey, Seth A. Faith, Tristan Booth, Resveratrol inhibition of Varicella Zoster Virus replication in vitro, Antiviral Res. 72 (2006) 171–177 [PMID 16899306].
[28] Pattaraporn Sasivimolphana, Vimolmas Lipipuna, Kittisak Likhitwitayawuid, Masaya Takemotoe, Pornpen Pramyothinc, Masao Hattori, Kimiyasu Shirakie, Inhibitory activity of oxyresveratrol on wild type and drug resistant Varicella-Zoster Virus replication in vitro, Antiviral Res. 84 (2009) 95–97 [PMID 19635502].
[29] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Epidemiol. 27 (3) (1938) 493–497.
[30] S. Kothandan, R. Swaminathan, Evaluation of in vitro antiviral activity of *Vitex negundo* L., *Hyptis suaveolens* (L) poit. *Decalepis hamiltonii* Wight &Arn., to Chikungunya virus, Asian Pac. J. Trop. Dis. 4 (2014) S111–S115.
[31] Julia Serkeidjiva, Stefka Ivancheva, Antiherpes virus activity of extracts from the medicinal plant *Geranium sanguineum* L, J. Ethnopharmacol. 64 (1) (1998) 59–68 [PMID 10075123].
[32] J.A. Erickson, M. Jalaie, D.H. Robertson, R.A. Lewis, M. Vieth, Lessons in molecular recognition: the effects of ligand and protein flexibility on molecular docking accuracy, J. Med. Chem. 47 (1) (2004) 45–55.
[33] Feixiong Cheng, Weihua Li, Yadi Zhou, Jie Shen, Zengrui Wu, Guixia Liu, Philip W. Lee, Yun Tang, AdmetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties, J. Chem. Inform. Modell. 52 (11) (2012) 3099–3105.
[34] H.J. O' Neil, J.D. Russell, D.E. Wyatt, P.V. McCaughey Coyle, Isolation of viruses from clinical specimens in microtitre plates with cells inoculated in suspension, J. Virol. Methods 62 (1996) 169–178 [PMID 9002075].
[35] T. O'Zaki, Y. Kajita, J. Namazue, K. Yamanishi, Isolation of Varicella-Zoster Virus from vesicles in children with varicella, J. Med. Virol. 48 (1996) 326–328 [PMID 8699164].
[36] C. Grose, P.A. Brunel, Varicella-Zoster Virus: isolation and propagation in human melanoma cells at 36 and 32 degrees C, Infect. Immun. 19 (1) (1978) 199–203 [PMID 203532].
[37] W.D. Rawlinson, D.E. Dwyer, V.L. Gibbons, A.L. Cunningham, Rapid diagnosis of Varicella-Zoster Virus infection with a monoclonal antibody based direct immunoflourescence technique, J. Virol. Methods 23 (1) (1989) 13–18 [PMID 2536379].
[38] M.M. Meqdam, D. Todd, M. Al-Abosi, Detection of Herpes Simplex and Varicella-Zoster Virus in clinical specimens using direct immunoflourescence and cell culture assays, Microbios 105 (2001) 111–118 [PMID 11393748].
[39] Vladimir N. Loparev, Takele Argaw, Philip R. Krause, Michiko Takayama, D. Scott Schmid, Improved identification and differentiation of Varicella-Zoster Virus (VZV) wild-type strains and attenuated Varicella Vaccine strain using a VZV Open Reading Frame 62-Based PCR, J. Clin. Microbiol. 38 (9) (2000) 3156–3160 [PMID 10970349].
[40] T.P. Lin, S.Y. Chen, P.D. Duh, L.K. Chang, Y.N. Liu, Inhibition of the Epstein-Barr virus lytic cycle by andrographolide, Biol. Pharm. Bull. 31 (11) (2008) 2018–2023.
[41] V. Pongthanapisith, K. Ikuta, P. Puthavathana, W. Leelamanit, Antiviral protein of Momordica charantia L. Inhibits different subtypes of Influenza A, Evid. Based Complement. Altern. Med. (2013) 1–7.
[42] Mohammed-Taghi Moradi, Ali Karimi, Somayeh Alidadi, Mostafa Gholami-Arjenaki, *In vitro* anti herpes simplex type-1 activity, anti-oxidant potential and total phenolic compounds of Pomegranate (*Punica granatum* L.) peel extract, J. Chem. Pharm. Res. 7 (8) (2015) 82–88.
[43] T. Lengauer, M. Rarey, Computational methods for biomolecular docking, Curr. Opin. Struct. Biol. 6 (3) (1996) 402–406 [PMID 8804827].
[44] D.B. Kitchen, H. Decornez, J.R. Furr, J. Bajorath, Docking and scoring in virtual screening for drug discovery: methods and applications, Nat. Rev. Drug Discov. 3 (11) (2004) 935–949 [PMID 15520816].
[45] Angamuthu Divyadarshini, Purushothaman Indu, Swaminathan Rajarajan, Ramachandran Sridhar, Screening of potential phytochemicals against protease of Varicella-Zoster Virus- an in silico approach, World J. Pharm. Pharm. Sci. 5 (5) (2016) 679–693.
[46] M. Pongracic, M. Mintas, S.R. Malic, Synthesis, kinetics, and molecular docking of novel 9-(2-hydroxypropyl) purine nucleoside analogs as ligands of herpes viral thymidine kinases, Helvetica chim. 85 (2002) 3237–3250.
[47] D. Shakya, A. Tiwari, M. Roy, P.K. Pal, P. Gangwar, Mohan, An *in silico* drug library search and docking studies with the target ORF 61gene product responsible for chickenpox, Int. J. Biol. Pharm. Allied Sci. 4 (6) (2015) 4236–4241.