Acta virologica **65**: 93 – 99, 2020 doi: 10.4149/av_2020_111

Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An *in-silico* approach

V. MATHIVADANI[1], A. S. SMILINE GIRIJA[2*], J. V. PRIYADHARSINI[3]

[1]II year BDS student, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai, Tamilnadu, India; [2]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P. H. Road, Chennai, Tamilnadu – 600077, India; [3]BRULAC-DRC, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai, Tamilnadu, India

Received February 26, 2019; revised June 10, 2019; accepted June 24, 2019

Summary. – Epstein-Barr virus (EBV), a B lymphotrophic herpesvirus associated with various forms of tumors, exhibits several latency phases with expressed EBV nuclear antigen 1 (EBNA-1). In the search of novel EBV-inhibiting targets, to curb the menace of EBV-borne lymphotropic transformations, EBNA-1 protein might serve as a best target for novel antiviral natural compounds. This study is thus aimed to explore the inhibitory potential of *Muuraya koengii* bioactive compounds isomahanine, murrayanol and mahanimbine against the EBNA-1 of EBV. 3D structure of EBNA-1 was retrieved from the PDB data bank with further optimization of both the protein and ligands. *In-silico* inhibitory potential of the selected *M. koengii* bio-compounds against EBNA-1 as well as the molecular properties of the derivatives against EBNA-1 were assessed. Murrayanol seems to be a potent inhibitory drug to target EBNA-1 with a promising binding energy of -7.21 with two hydrogen bonds. Drug likeliness parameters recorded murrayanol to be the most promising of the tested compounds, followed by isomahanine. Molecular docking evaluations show that EBNA-1 might be inhibited with *M. koengii* biocompounds.

Keywords: EBV; EBNA; M. koengii; in-silico

Introduction

Epstein-Barr virus (EBV), commonly referred as human herpesvirus 4 (HHV-4) and belonging to the *Herpesviridae* family manifests asymptomatic acute infection in immunocompetent children or infectious mononucleosis in older patients and is spread by the oral transfer of saliva. The virus is associated with B and T cell lymphomas and nasopharyngeal carcinomas (Odumade *et al.,* 2011). EBV initiates an efficient infection in B lymphocytes mediated by the interactions between the viral envelope gp350 with the complement receptor CD21 (Shannon-Lowe *et al*., 2011). During the lytic and latent stages, many viral proteins play a critical role in viral fusion, replication or B cell transformation. Emerging from the early regulatory proteins and early antigens, the EBV nuclear antigens (EBNAs) and the latent membrane proteins characterize the protein expression of the latent phase. EBNAs encompass six proteins (EBNA-1–6), and EBNA-1 is involved in the initial phase of maintaining the episomal state of EBV DNA with further activation of the other EBNA proteins towards transforming the B lymphocytes (Leight *et al.,* 2000).

Chemically, EBNA-1 is a multifunctional, dimeric viral protein with glycine-alanine repeat sequence separating the protein into amino- and carboxy-terminal domains. Episomal formation involves a dyad symmetry of four EBNA-1 sequence-specific binding sites leading to the

*Corresponding author. E-mail: smilinejames25@gmail.com; phone: +91-9841516172 (mobile).

Abbreviations: EBV = Epstein-Barr virus; EBNA-1 = EBV nuclear antigen 1

progression of viral replication aided by 20-site repeat segments. In addition, the sequence is expressed from the Qp promotor and impairs the antigen processing and MHC class I-restricted antigen presentation, thereby inhibiting the CD8-restricted cytotoxic T cell response against virus-infected cells (Callan *et al.,* 1998). In addition, EBNA is considered as a potent marker in distinguishing the virus-mediated cancer cells from normal cells and is vital for the persistence of the virus in host cells (Wilson *et al.,* 2018).

Routine antiviral agents are considered as less effective in the initial phase of EBV infection. In recent years, targeting EBNA-1 sheds light on curbing EBV replication as it is involved with the transcriptional regulation of the viral replication (Delecluse *et al.,* 1994). In this concern, alternative therapeutics with the bioactive viral compounds from medicinal plants and herbs have spurred renewed interest in recent years. The leaves of *Murraya koengii*, a tropical to sub-tropical tree in the family *Rutaceae*, commonly referred as "curry" leaves is used for culinary purposes. It has also wide application in Ayurvedic and Siddha medicine for its potent bioactive compounds like cinnamaldehye and numerous carbazole alkaloids, including mahanimbine and girinimbine with anti-viral effects (Al Harby *et al.*, 2016). The role of *M. koengii* leaves has also been associated with the stabilization of its effect with silver nanoparticles (Christensen *et al.,* 2011), antimicrobial effects (Rahman and Gray, 2005) and anti-tumor effect against the expression of early antigens of EBV in cell lines (Handral *et al.,* 2008). With this background, this study is aimed at targeting the EBNA-1 by isomahanin, murrayanol and mahanimbine from *M. koengii* leaves using an *in-silico* approach.

Materials and Methods

Retrieval of SAP and protein optimisation. The 3D crystal structure of Epstein Barr virus nuclear antigen – 1 was retrieved from RCSB protein data bank (http://www.rcsb.org/pdb). Hydrogen atoms, solvation parameters and fragmental volumes to the protein were added and electronic charges were assigned to the protein atoms using kollman united atoms force field by using Auto Dock Tool (ADT) –2.0.

Ligand preparation and optimisation. Using Chemsketch software the structures of isomahanine, murrayanol, and mahanimbine and the control acyclovir were drawn together with the generation of their 3-D structures and optimization. The selected ligands were retrieved in SDB format, which were further saved in.mol file followed by the subsequent conversion using open babel molecular converter program (Boyle *et al.,* 2011) and were saved in PDB format. Molinspiration assessment of the molecular properties of the selected compounds: The physicochemical and the pharmacological properties such as logP, hydrogen bond donor and acceptor characteristics, molecular size and rotatable bonds were predicted by molinspiration server (Jarrahpour *et al.,* 2010). Based on the Lipinsky's rule of five (Lipinski *et al.,* 2001) characterization of the absorption, distribution, metabolism and elimination (ADME) of the selected compounds with further assessments and estimations of the molecular properties of the selected ligands was assessed. Membrane permeability and bio-availability was also evaluated.

Docking simulations and interpretations. The docking analysis to interpret the affinity between isomahanine, murrayanol, and mahanimbine and the control acyclovir against EBNA-1 was achieved by auto-dock tool (Molecular Graphics Laboratory, USA) with the intermediary steps such as pdb.qt files for the proteins and the ligands. Using graphical user interface program Auto-Dock tool (ADT) the grid box creation was completed. Prior preparation of the grid map using the grid box with a grid size of 126x126x126 xyz points was done. Further, using Lamarckian genetic algorithm (LGA), docking simulation was achieved by setting the initial position, orientation and torsions of the ligand molecules in a random position. 10 different runs set to terminate after a maximum of 25,0000 energy evaluations was used for each docking experiment with the population size set at 150. A translational step of 0.2 Å, quaternion and torsion steps of 5 were applied for each dock. The most favorable free energy of binding is achieved by clustering the results >1.0 Å in positional root-mean-square deviation (RMSD) (Blum *et al.,* 2008). Finally, the pose was extracted and aligned with the receptor structure with the lowest binding energy or binding affinity for final analysis.

Docking visualisation. The protein-ligand interactions like hydrogen bonding and other non-bonded energies between the isomahanine, murrayanol and mahanimbine and the control acyclovir against EBNA-1 were visualized using PYMOL software. The relative stabilities were evaluated using their molecular dynamics, binding affinities, energy simulations with further docking score assessments.

Results

Structure retrieval of the EBNA-1 protein of EBV

The crystal structure of hexameric ring of EBNA-1 obtained from EBV (strain B95-8) is downloaded from PDB database and its and its structure ID was documented as 5WMF-A-Chain (Fig. 1). Removal of the water molecules and final stage merging of hydrogen atoms to the receptor molecule was successful. The 3D structure of EBNA-1 was visualized using RASMOL with the analysis of pink color indicating the alpha-helix, yellow arrow indicating the beta sheets and white color indicating the turns (Fig. 2).

Fig. 1

Crystal structure of the ligand EBNA-1 from PDB

Fig. 2

RASMOL 3D structure of EBNA-1

Table 1. 2D and 3D structures and SMILES format of the selected bio-compounds

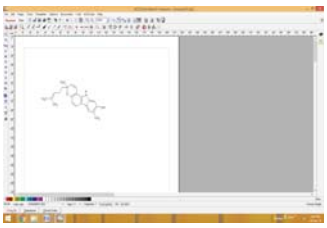
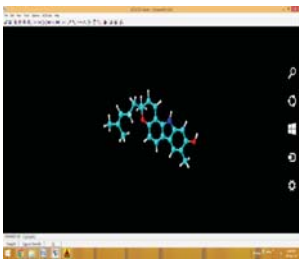
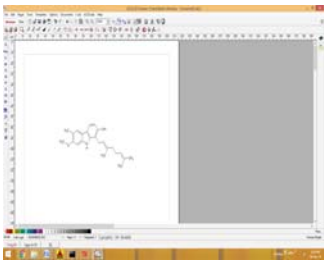
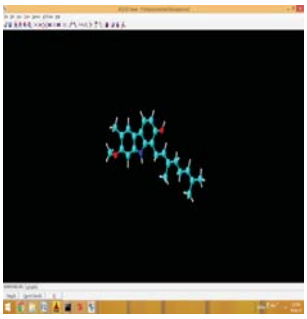
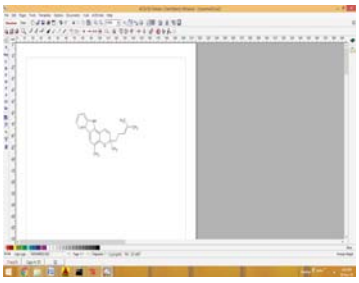
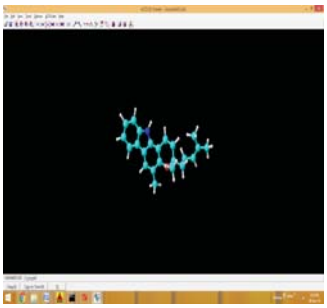
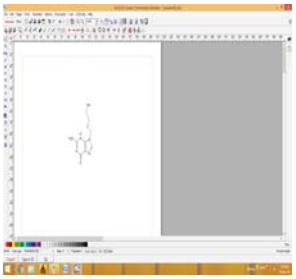
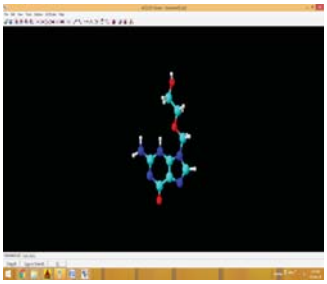
Compounds	2D	3D	SMILES
Isomahanine			CC1=C(C=C2C(=C1)C3=C(N2)C4=C(C=C3)OC(C=C4)(C)CCC=C(C)C)O
Murrayanol			CC1=C(C=C2C(=C1)C3=C(N2)C(=C(C=C3)O)CC=C(C)CCC=C(C)C)OC
Mahanimbine			CC1=C2C(=C3C(=C1)C4=CC=CC=C4N3)C=CC(O2)(C)CCC=C(C)C
Acyclovir			C1=NC2=C(N1COCCO)NC(=NC2=O)N

Table 2a. Molinspiration calculations of *M. koengii* bio-compounds

Compounds	M. wt	Mol formula	Hydrogen Bond Donor	Hydrogen Bond Acceptor	miLogP	Rotatable bonds	Viola-tions	TPSA (Å)	Volume	N atoms
Isomahanine	347.458	$C_{23}H_{25}NO_2$	2	3	7.07	3	1	45.25	334.36	26
Murrayanol	363.501	$C_{24}H_{29}NO_2$	2	3	7.47	6	1	45.25	362.06	27
Mahanimbine	331.459	$C_{23}H_{25}NO$	2	1	7.10	3	1	25.02	326.34	25
Acyclovir	225.208	$C_8H_{11}N_5O_3$	8	4	-1.61	4	0	119.06	187.75	16

Table 2b. Drug likeliness of *M. koengii* bio-compounds

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Isomahanine	0.16	0.13	1.19	0.65	0.03	0.41
Murrayanol	0.17	0.05	0.15	0.56	-0.18	0.43
Mahanimbine	0.16	0.13	0.16	0.57	0.05	0.39
Acyclovir	-0.11	-0.05	0.04	-1.35	-0.77	0.84

Structure retrieval of the M. koengii compounds (the ligands)

The ligand optimization was achieved using ACD Chemsketch and retrieved in a compatible format using Open Babel molecular converter tool. The retrieved 2D and 3D structures of the ligands and its SMILES format are shown in Table 1.

Molinspiration estimation towards drug likeliness

The bioactivity prediction scores of isomahanine, murrayanol, mahanimbine and the control acyclovir against EBNA-1 of EBV, based on the drug likeness calculations, are scored and tabulated in Table 2a and 2b. Molecular properties were calculated based on the Lipinski's rule of five. From the molinspiration results and the n-violation values not satisfying the Lipinski's Rule of 5, the bioactive compounds isomahanine, murrayanol and mahanimbine had 1 violation each, whereas the control drug acyclovir showed 0 violations. TPSA was < 140 Å for all the compounds. Drug likeliness scored high for isomahanine and murrayanol for nuclear ligand receptor and for kinase activities when compared to acyclovir.

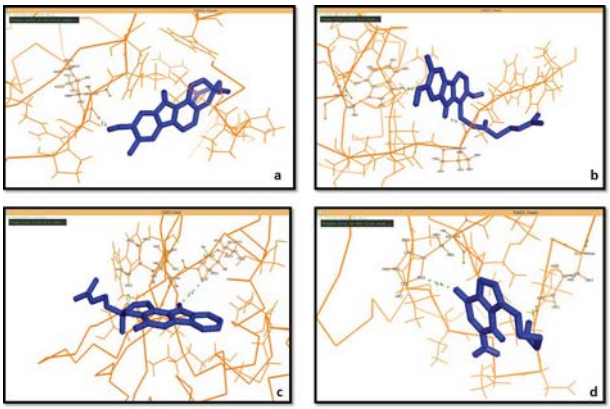


Fig. 3

PYMOL visualisation of the hydrogen interactions between *EBNA-1* and (a) Isomahanine, (b) Murrayanol, (c) Mahanimbine, (d) Acylovir

Table 3. Overall docking results of *M. koengii* bio-compounds with EBNA-1 of EBV

Docking analysis of EBNA 1 with	Number of hydrogen bonds	Binding energy	Ligand efficiency	Inhibition constant (μM)	Inter molecular energy	vdW + Hbond + desolv Energy	Electro-static energy	Torsional energy	Total internal unbound
Isomahanine	1	-7.16	-0.28	5.69	-8.35	-8.32	-0.03	1.19	-0.72
Murrayanol	2	-7.21	-0.27	5.16	-9.3	-9.25	-0.05	2.09	-0.74
Mahanimbine	2	-6.33	-0.25	22.76	-7.23	-7.12	-0.11	0.89	-0.34
Acyclovir	2	-3.67	-0.23	2.05	-5.46	-4.17	-1.29	1.79	-1.94

Docking analysis of the M. koengii compounds against EBNA-1

The best conformers were selected using LGA based on the best ligand-receptor structure from the docked structure based on the lowest energy and minimal solvent accessibility. PYMOL visualizing tool of the hydrogen bond interactions in stick model between the isomahanine, murrayanol and mahanimbine and the control acyclovir against EBNA-1 is shown in Fig. 3. The amino acids of EBNA-1 binding with the bioactive compounds, namely, isomahanine, murrayanol and mahanimbine showed the binding energy of -7.16 Kcal/mol with one hydrogen bond interaction, -7.21 Kcal/mol with 2 hydrogen bond interactions, -6.33 Kcal/mol with two hydrogen bond interactions, respectively. The compounds utilized lesser energy than acyclovir control with -3.67 Kcal/mol with two hydrogen bond interactions. The torsional energy and the docking scores between the drug and ligands are shown in Table 3. The docking results show *M. koengii* bio-compounds have better binding energy and bonding with the target receptor EBNA-1 in comparison with acyclovir. It was also evident that compound murrayanol was more potent in targeting EBNA-1.

Discussion

Epstein-Barr virus nuclear antigen 1 plays a role in varying the extent in EBV-associated tumors (Kieff *et al.,* 1990). Failure of the clinical success with the prevailing antiviral drugs leads to the search of novel target to combat the EBV entry, fusion and replication. In this context, EBNA-1 can be considered as a good target for novel antiviral agents. *M. koengii* and its bio-compounds, being phytochemically rich in tannins, flavonoids and alkaloids, are known for their antimicrobial potential (Bhandari, 2012). The good fit of the bio-compounds with the EBNA-1 was efficiently achieved in the present study by molecular docking analysis. Of several forms of EBNA proteins, EBNA-1 was retrieved from the PDB database as a desirable target based on the data recorded in database and was freely accessible. In the present study, isomahanine, murrayanol and mahanimbine and the control acyclovir were docked against EBNA-1, resulting in a promising receptor-ligand complex.

Amidst various antiviral drugs, acyclovir is the routine antiviral drug of choice for herpesviruses, thus we included it same as a control to compare the selected *M. koengii* bio-compounds. Docking analysis involves two major steps to predict orientation (pose) and binding energy by scoring (Wang, 2003). We used standard docking experiments using auto-dock 4.2 tool, which is a suite of automated docking tools with a software for modelling flexible small molecule by employing a rigid receptor/flexible ligand protocol, while exploring conformational space within a specified grid box designated by the user. A successful re-docking will be within 2 angstroms of the experimentally known site and corresponds to one of the top ranked binding energies (Huang and Zou, 2006; Bikadi and Hazai, 2009). In addition, the sum of the energy of ligand and receptor separately is greater than the total energy when bound together, and the difference is considered as the binding free energy. A higher negative energy indicates a deeper potential energy well, a more stable complex, and more likely binding mode (Huey *et al.,* 2007). In this context, the Lamarckian Genetic Algorithm (LGA) was used to explore the binding conformational landscape of isomahanine, murrayanol and mahanimbine and the control acyclovir docked against EBNA-1. The docking scores on EBNA-1 indicated that there is a direct relationship between the energy of the binding affinity, referring to the lowest docking scores, and the stability. In accordance with this, apart from the binding energy, the inter-molecular energy, van-der-Waal's energy and torsional energy were also at a higher end for murrayanol followed by isomahanine.

Analysis using PYMOL visualizer to predict hydrogen bond interactions between EBNA-1 and the ligands yielded promising results with hydrogen bonds and bonding energies. The number of hydrogen bonds together with the enthalpic gain due to the water molecules determines the best fit (Clarke *et al.,* 2001). In this context, murrayanol scores to be the best inhibitory agent of EBNA-1 with a highest docking score of -7.21 Kcal/mol, but showed only one hydrogen bond. However, acylovir showed a higher energy of -3.67 Kcal/mol, albeit 2 hydrogen bonds, similar to the *M. koengii* compounds. Isomahanine seems to be the next successful candidate in targeting the EBNA-1.

We performed molinspirational calculations in the present study to assess and evaluate the drug likeliness of the selected ligands. This is due to the fact that molecular properties such as membrane permeability, hydrophobicity and bioavailability are associated with some basic molecular descriptors such as log P (partition coefficient), log S (solubility), molecular weight, number of hydrogen bond acceptors and donors in a molecule. They are attributed to the concept of drug likeliness of the ligands, which has wide acceptance in novel drug discovery and development (Leeson *et al.,* 2007). In the present study, though the n-violation values of the selected bioactive compounds with isomahanine, murrayanol and mahanimbine were 1 and for the control acyclovir was zero, we proceeded with docking analysis as they showed a good drug likeliness score.

In molinspiration analysis, topological polar surface area (TPSA) of a molecule is considered as a useful descriptor to characterize the drug absorption and bio-availablity and the values of TPSA and OH-NH interactions indicate that the selected ligands viz, isomahanine, murrayanol, and mahanimbine, possess a smooth and efficient binding to the target proteins. In addition, the bioactive compounds with TPSA values of >140 Å or higher are known to possess low-absorption in association with the lipophilicity (miLogP) values. In this context, all tested ligands score high absorption with high membrane penetration with a TPSA score of <140 Å suggesting the oral bio-availability of the selected compounds. However, the study has its limitations as the molecular docking evaluations are theoretical evidence on the bio-activity of *M. koengii* compounds and requires further experimental validations.

Conclusion

Selection of novel inhibitors specific against EBNA-1 protein by computational assessments has spurred renewed interest in recent years. The docking calculations in this study suggest the promising inhibitory effect of isomahanine, murrayanol, and mahanimbine from *M. koengii* against the EBNA-1. The preliminary clue obtained from the present investigation provides first information for further target-based experimental screening of the *M. koengii* bio-compounds for better selectivity and mechanism of action.

References

Al Harbi H, Irfan U, S Ali S (2016): The antibacterial effect of Curry Leaves (Murraya Koenigii). Eur. J. Pharm. Med. Res. 3, 382–387.

Bhandari PR (2012): Curry leaf (Murraya koenigii) or Cure leaf: Review of its curative properties. J. Med. Nutr. Nutraceut. 1, 92–97 https://doi.org/10.4103/2278-019X.101295

Bikadi Z, Hazai E (2009): Application of the PM6 semi-empirical method to modelling proteins enhances docking accuracy of AutoDock. J. Cheminform. 1, 15. https://doi.org/10.1186/1758-2946-1-15

Blum C, Blesa MJ, Roli A, Sampels M (2008): Hybrid Metaheuristics: An Emerging Approach to Optimization. Studies in Computational Intelligence. Berlin Heidelberg : Springer-Verlag, p. 114. https://doi.org/10.1007/978-3-540-78295-7

Boyle ONM, Banck M, James CA, Morley C, Vandermeersch T et al. (2011): Open Babel An open chemical toolbox. J. Cheminform. 3, 33–36. https://doi.org/10.1186/1758-2946-3-33

Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA, Steven N, McMichael AJ, Rickinson AB (1998): Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus in vivo. J. Exp. Med. 187, 1395–1402. https://doi.org/10.1084/jem.187.9.1395

Christensen L, Vivekanandhan S, Misra M, Mohanty AK (2011): Biosynthesis of silver nanoparticles using murraya koenigii (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. Adv. Mater. Lett. 2, 429–434. https://doi.org/10.5185/amlett.2011.4256

Clarke C, Woods RJ, Gluska J, Cooper A, Nutley MA, Boons GJ (2001): The Effect of Water Displacement on Binding Thermodynamics: Concanavalin A. J. Am. Chem. Soc. 123, 12238–12247. https://doi.org/10.1021/ja004315q

Delecluse HJ, Bartnizke S, Hammerschmidt W, Bullerdiek J, Bornkamm GW (1993): Episomal and integrated copies of Epstein-Barr virus coexist in Burkitt lymphoma cell lines. J. Virol. 67, 1292–1299. https://doi.org/10.1128/JVI.67.3.1292-1299.1993

Huang SY, Zou X (2006): Efficient molecular docking of NMR structures: Application to HIV-1 protease. Protein Sci. 16, 43–51. https://doi.org/10.1110/ps.062501507

Huey R, Morris GM, Olson AJ, Goodsell DS (2007): A semiempirical free energy force field with charge-based desolvation. J. Comput. Chem. 28, 1145–1152. https://doi.org/10.1002/jcc.20634

Jarrahpour A, Fathi J, Mimouni M, Hadda TB, Sheikh J, Chohan Z et al. (2012): Petra, Osiris and Molinspiration (POM) together as a successful support in drug design: Antibacterial activity and biopharmaceutical characterization of some azo schiff bases. Med. Chem. Res. 21, 1–7. https://doi.org/10.1007/s00044-011-9723-0

Kieff E, Liebowitz D (1990): Epstein-Barr virus and its replication. In Fields BN, Knipe DM (Eds): Virology. New York : Raven Press, pp. 1889–1920.

Leeson PD, Springthorpe B (2007): The influence of drug-like concepts on decision-making in medicinal chemistry. Nat Rev Drug Discov. 6, 881–890. https://doi.org/10.1038/nrd2445

Leight ER, Sugden B (2000): EBNA-1a protein pivotal to latent infection by Epstein-Barr virus. Rev. Med. Virol. 10, 83–100. https://doi.org/10.1002/(SICI)1099-1654(200003/04)10:2<83::AID-RMV262>3.0.CO;2-T

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001): „Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Stings." Adv. Drug. Delivery Rev. 46, 3–26. https://doi.org/10.1016/S0169-409X(00)00129-0

Odumade OA, Hogquist KA, Balfour HH Jr (2011): Progress and problems in understanding and managing primary Epstein-Barr virus infections. Clin. Microbiol. Rev. 24, 193–209. https://doi.org/10.1128/CMR.00044-10

Rahman MM, Gray AI (2005): A benzoisofuranone derivative and carbazole alkaloids from Murraya koenigii and their antimicrobial activity. Phytochemistry 66, 1601–1606. https://doi.org/10.1016/j.phytochem.2005.05.001

Shannon-Lowe C, Rowe M (2011): Epstein-Barr Virus Infection of Polarized Epithelial Cells via the Basolateral Surface by Memory B Cell-Mediated Transfer Infection. PLoS Pathog. 7, e1001338. https://doi.org/10.1371/journal.ppat.1001338

Handral HK, Pandith A and Shruthi SD (2012): A review on Murraya koenigii: multipotential medicinal plant Asian J. Pharm. Clin. Res. 5, 5–14.

Wang R, Lu Y, Wang S (2003): Comparative evaluation of 11 scoring functions for molecular docking. J. Med. Chem. 46, 2287–2303. https://doi.org/10.1021/jm0203783

Wilson JB, Manet E, Gruffat H, Busson P, Blondel M, Fahraeus R (2018): EBNA1: Oncogenic Activity, Immune Evasion and Biochemical Functions Provide Targets for Novel Therapeutic Strategies against Epstein-Barr Virus-Associated Cancers. Cancers (Basel) 10, 109–113. https://doi.org/10.3390/cancers10040109