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Research Article

DISCOVERY OF A NOVEL BINDING TRENCH IN BMRF1 OF EPSTEIN BARR VIRUS AN *IN SILICO* APPROACH

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ABSTRACT

Objective: A few number of *in vitro* and *in vivo* experimental is underway in identifying an effective drug against Epstein Barr virus. This study is an attempt in identifying an effective drug against this virus by *in silico* analysis of more than 100 bioactive compounds derived from plants. The BMRF1 gene is a DNA polymerase processivity factor that has a prime role in lytic replication and DNA synthesis.

Methods: The physical, chemical and molecular characteristics of each compound were studied by Chemoinformatics tools and docked against BMRF1 gene.

Result: From this study, it was found that Astragaloside-V and Astragaloside-III showed the best interaction value against BMRF1 protein.

Conclusion: Further analysis of these compounds against this protein will lead a new pathway to drug discovery.

Keywords: Epstein Barr virus, iGemdock, *in silico* analysis, BMRF1, DNA polymerase processivity factor.

INTRODUCTION

Epstein barr virus (Human Herpesvirus -4), described as the most common virus affecting human.It mostly causes glandular fever and certain type of cancers namely Hodgkin's lymphoma, Burkitt's lymphoma and nasopharyngeal carcinoma (NPC). About 95% of the world population is found to be infected between age 35 and 40 as given by Centre for Disease Control and Prevention [1,2].

The virus belongs to human lymphotrophic gamma herpesvirus family [3] and its genome is made up of 85 genes of size 1920 bp in length. The latency gene, EBNA1 is constantly expressed in all forms of viral – associated tumors and latency. It is the only protein expressed during latent and lytic cycle of infection .The crystal structure of the homo-dimeric EBNA1 of 18bp binding site was solved at 2.4 A resolution. It is found to be made up of two domains, flanking domain along with a helix that enters into a major groove and an extended chain that pass through the minor groove in order to keep in contact all the sequences with the DNA. While the second domains, core domain does not make any direct contact with the DNA [4].

The EBNA1 plays an important role in establishing prolonged survival of latently infected cells and latent episomal infection. The transcription of viral and cellular genes is regulated by EBNA1 stimulating the DNA replication at OriP (viral origin of plasmid replication). Hence, EBNA1 is termed as a multifunctional DNA binding protein [5].It was also shown that EBNA1 plays an important role in the reactivation of the virus from the latency stage to the lytic stage in epithelial cells by depleting in latently infected cells that paces way to the reactivation of the Epstein barr virus. Thus, it confirms that reactivation of the virus is suppressed by EBNA1. The depletion of EBNA1will result in the reduced expression of lytic gene and amplification of DNA which demonstrates that it completely involved in the lytic infection [6]. It has also been established that EBNA1 plays a vital role in the replication and maintenance of the virus [7]. In vitro studies had revealed that it works as a strong RNA binding protein by interrelating with different substrates, EBV- encoded RNA polymerase III transcript, EBER1 and the HIV-encoded transactivation response (TAR) element [8].

Proteomic profiling was carried out to study the EBNA1-host protein interaction in 293T and HeLa cells during latent and lytic infection. It was found that these interactions occur in nasopharyngeal carcinoma, gastric carcinoma cells, nucleophosmin and specific RNA binding proteins that are present during latent and lytic infection [9]. The dynamic change that occurs in a MicroRNA profile expression resulting in the development of nasopharyngeal carcinoma was studied by *in silico* analysis. It was found that miR-29a/c, miR-34b, miR-34c-3p, miR-34c-5p, miR-429, miR-203, miR-222, miR-1/206, miR-141, miR-18a/b, miR-544, miR-205 and miR-149 played an important role in the progress of NPC and transcription factors such as ETS2, MYB, Sp1, KLF6, NFE2, PCBP1 and TMEM54 was involved in the regulatory functions in the expression of miRNA [10].

The crystal structure of BMRF1, DNA polymerase processivity factor was solved in an oligomeric state. It was revealed that protein structure varied from a monomer to a trimer, C-shaped head to head dimer took part in the interface with the DNA [11]. It is found to be the major early phosphoprotein involved in the lytic replication and vital for DNA synthesis carried out by BALF5 polymerase catalytic subunit [12,13]. The early promoter, BHLF1 present in the lytic origin of viral DNA replication, oriLyt was transciptionally activated by BMRF1 [14]. The initial transcriptional replication complexes required for transcription process was developed by the interaction between BMRF1 protein and its promoter-bound cellular transcriptional factors, ZBP-89 and/or SP1 [15]. The regulation of EA-D (BMRF1) promoter by the two immediate-early viral transactivators, BZLF1 and BRLF1 was found to be cell type specific [16].

In this paper, we have studied the physical, chemical and molecular properties of bioactive compounds of plant source and compounds exhibiting anti-inflammatory properties. These compounds were docked against BMRF1, DNA polymerase processivity factor gene present in the Epstein Barr virus.

MATERIALS AND METHODS

Protein and compounds of interest

The structure of the protein of interest, BMRF1, DNA polymerase processivity factor of PDB ID 2Z0L was retrieved from Protein databank.

Prediction of its antigenic value, its subcellular location, isoelectric point and Aliphatic index

The antigenicity and the subcellular location of the protein were predicted by VaxiJen v2.0 (0.5% threshold) and Virus-pLoc respectively. The isoelectric point and aliphatic index were calculated using CLC bio workbench.

Selection of bioactive compounds

More than 20 plants were selected based on its medicinal value for this study (Table 1). The structures of the compounds were retrieved from Dr. Duke's phytochemical and ethnobotanical database. (Http: / http://www.ars-grin.gov/duke/) and PubChem project (http://pubchem.ncbi.nlm.nih.gov/).

Screening of compounds

The molecular properties such as LogP, number of hydrogen bond donors and acceptors and polar surface area were calculated using Molinspiration, a web based cheminformatics tool (www.molinsipiration.com). Next, the compounds were screened for its toxicity, Ames test and rodent carcinogenicity assay using the PreADMET tool (http://preadmet.bmdrc.org).

Docking studies

Various ligands were docked against BMRF1 protein using iGemdock (http: gemdock.life.nctu.edu.tw/dock). Computing of ligand conformation and orientation is carried out based on the active site of the target protein.

RESULT AND DISCUSSION

The primary role of BMRF1 protein is DNA polymerase processivity involved in the replication of DNA viruses and hence the docking study was designed to target BMRF1 gene. The sub cellular location of the BMRF1 protein of 10 different strains using Virus-pLoc was found to be in the host nucleus. The antigenic value, pI and aliphatic index of the 10 strains were found to be similar (Table1).

Totally 164 compounds were considered for this study out of which ninety one were plant derived compounds (Table 2, 3). These compounds were subjected to initial screening through Molinspiration tool where the molecular properties and the prediction of the bioactive score for the most significant drug target of each compound were analyzed. Out of 164 compounds, 142 compounds passed the Lipinski rule of five which is the basis of the molinspiration tool. The molecular property of each compound is determined by this rule. The properties like adsorption, distribution, metabolism and excretion (ADME) are studied by this rule.

The compounds which have cleared molinspiration test were further subjected to Ames test and carcinogenicity test in mouse and rat through Preadmet tools. Twenty two compounds were found to be non mutagenic and non carcinogenic in rat and mouse (Table 4). The total of twenty two compounds was docked against BMRF1 protein using iGEMDOCK to understand is interactive analysis. Astragaloside-V (-149.06) exhibited the highest interaction with BMRF1 protein followed by Astragaloside-III (0923)-136.85, Astragaloside-IV (-130.33), Astragaloside-III (-129.22) and Astragaloside-VII (-105.59) (Table 5; Figure 1). The hydrogen bond and Vander Waal bond interaction were also analyzed. Hydrogen bond interaction was found in Glu-2, Thr-3. Thr-4, Tyr-114, Lys-115, Arg-116, Gln-118, Phe-113 and Glu-124 (Table 6) and Vander Waal bond interaction was seen with Glu-2, Thr-3, Lys-115, Arg-116, Pro-117, Tyr-114 and Phe-125 (Table 7).

Table 1: The antigenic value, subcellular location with pI and Aliphatic index of Epstein barr Virus BMRF1 gene

Accession number	Antigenic value	Location	pI	Aliphatic index
AJ507799	0.6037	Host nucleus	9.19	81.91
NC007605	0.6037	Host nucleus	9.19	81.91
AY961628	0.5897	Host nucleus	9.19	81.19
DQ279927	0.6037	Host nucleus	9.19	81.91
KC207814	0.6037	Host nucleus	9.19	81.91
KC207813	0.6037	Host nucleus	9.19	81.91
HQ020558	0.6090	Host nucleus	9.19	81.66
V01555	0.6037	Host nucleus	9.19	81.91
JQ009376	0.5952	Host nucleus	9.19	81.66
NC009334	0.6037	Host nucleus	9.19	81.91

Table 2: List of potential bioactive compounds identified from medicinal important plants selected for docking studies against Epstein barr virus BMRF1 gene

Plant source	Compounds	Molinspiration	Molecular formula	Molecular weight
Brassica juncea	Diallyl sulfide	+	$C_6H_{10}S$	114.21
	allyl methyl disulfide	+	$C_4H_8S_2$	120.24
	butyl isothiocyanate	+	C_5H_9NS	115.20
	Pentyl isothiocyanate	+	$C_6H_{11}NS$	129.22
	methylallyl trisulfide	+	$C_4H_8S_3$	152.30
	3-Phenylpropionitrile	+	C_9H_9N	131.17
	diallyl trisulfide	+	$C_6H_{10}S_3$	178.34
	6-Undecanol	+	$C_{11}H_{24}O$	172.31
	phenethyl isothiocyanate		C_9H_9NS	163.24
	diallyl tetrasulfide	+	$C_6H_{10}S_4$	210.40
	Bisallylthiocarbamide	+	$C_7H_{12}N_2S$	156.25
	Allyl isothiocyanate	-	C_4H_5NS	99.15
	Crotonyl isothiocyanate	-	C_5H_5NOS	127.16
Thuja orientalis	Tricyclene	+	$C_{10}H_{16}$	136.23
	alpha-pinene	+	$C_{10}H_{16}$	136.23
	beta-pinene	+	$C_{10}H_{16}$	136.23
	beta-myrcene	+	$C_{10}H_{16}$	136.23
	Delta-3-carene	+	$C_{10}H_{16}$	136.23
	beta.-Phellandrene	+	$C_{10}H_{16}$	136.23
	gamma-terpinene	+	$C_{10}H_{16}$	136.23
	Terpinolene	+	$C_{10}H_{16}$	136.23
	Terpinene-4-ol	+	$C_{10}H_{18}O$	154.25
	alpha-terpineol	+	$C_{10}H_{18}O$	154.25
	bornyl acetate	+	$C_{12}H_{20}O_2$	196.29
	Thujopsene	+	$C_{15}H_{24}$	204.35
	Elemol	+	$C_{15}H_{26}O$	222.37

	Cedrol	+	$C_{15}H_{26}O$	222.37
	Alpha-Thujene	+	$C_{10}H_{16}$	136.23
	alpha-Fenchene	+	$C_{10}H_{16}$	136.23
	sabinene	+	$C_{10}H_{16}$	136.23
	alpha phellandrene	+	$C_{10}H_{16}$	136.23
–	limonene	+	$C_{10}H_{16}$	136.23
	linalool	+	$C_{10}H_{18}O$	154.25
	thymoquinone	+	$C_{10}H_{12}O_2$	164.20
	geranyl acetate	+	$C_{12}H_{20}O_2$	196.29
	beta-Cedrene	+	$C_{15}H_{24}$	204.35
	alpha.-Cadinol	+	$C_{15}H_{26}O$	222.37
Nerium indicum	odorinol	+	$C_{18}H_{24}N_2O_3$	316.40
	Pregnenolone	+	$C_{21}H_{32}O_2$	316.48
	oleandrigenin	+	$C_{25}H_{36}O_6$	432.55
	Adynerigenin	+	$C_{23}H_{32}O_4$	372.50
Pratinia villosa	Luteolin	+	$C_{15}H_{10}O_6$	286.27
	Apigenin	+		
Citrus unshiu	Gardenin	+	$C_{21}H_{22}O_9$	418.40
	Umuhengerin	+	$C_{20}H_{20}O_8$	388.37
Adanosonia digitata	epicatechin	+	$C_{15}H_{14}O_6$	290.27
	Astraisoflavan	+	$C_{23}H_{28}O_{10}$	464.46
	Cycloastragenol	-	$C_{30}H_{50}O_5$	490.71
	Cycloastragenol (2)	-	$C_{30}H_{50}O_5$	490.71
	Astrapterocarpan	-	$C_{17}H_{16}O_5$	300.30
	Betaine	+	$C_5H_{11}NO_2$	117.15
	Choline	+	$C_5H_{14}NO^+$	104.17
	Formononetin	+	$C_{16}H_{12}O_4$	268.26
	Gaba	-	$C_4H_9NO_2$	103.12
	isoliquiritigenin	+	$C_{15}H_{12}O_4$	256.25
	Astragaloside -I	+	$C_{41}H_{68}O_{14}$	784.97
	Astragaloside –II	+	$C_{42}H_{68}O_{16}$	828.98
	Astragaloside-II (0922)	-	$C_{41}H_{66}O_{15}$	798.95
	Astragaloside-III	+	$C_{41}H_{68}O_{14}$	784.97
	Astragaloside-III (0923)	+	$C_{41}H_{68}O_{14}$	784.97
	Astragaloside-IV	+	$C_{41}H_{68}O_{14}$	784.97
	Astragaloside-V	+	$C_{47}H_{78}O_{19}$	947.11
	Astragaloside-VI	-	$C_{47}H_{78}O_{19}$	947.11
	Astragaloside-VII	+	$C_{47}H_{78}O_{19}$	947.11
	Astramembrangenin (1)	+	$C_{30}H_{50}O_5$	490.71
	Astramembrangenin(2)	+	$C_{30}H_{50}O_5$	490.71
Brassica armeniaea	Allyl-Isothiocyanate	+	C_4H_5NS	99.15
	Crotonyl isothiocyanate	+	C_5H_5NOS	127.16
TUSSILAGO FARFARA	2-O-Methyl-d-xylose	+	$C_6H_{12}O_5$	164.16
	Quercetin	+	$C_{15}H_{10}O_7$	302.23
Bergenia ligulata	Berginin	+	$C_{14}H_{16}O_9$	328.27
	Afzelechin	+	$C_{15}H_{14}O_5$	274.27
	Gallic acid	+	$C_7H_6O_5$	170.12
	parasorbic acid	+	$C_6H_8O_2$	112.13
	isovaleric acid	+	$C_5H_{10}O_2$	102.13
	Terpinen-4-ol	+	$C_{10}H_{18}O$	154.25
	(Z)- asarone	+	$C_{12}H_{16}O_3$	208.25
Eugenia jambola	Kaempferol	+	$C_{15}H_{10}O_6$	286.24
	Ellagic acid	+	$C_{14}H_6O_8$	302.19
	Mycaminose	+	$C_8H_{17}NO_4$	191.22
	Anthocyanins	+	$C_{15}H_{11}O^+$	207.25
	petunidin	-	$C_{16}H_{13}ClO_7$	352.72
	Myrtenol	+	$C_{10}H_{16}O$	152.23
	Eucarvone	+	$C_{10}H_{14}O$	150.22
	(+)-T-muurolol	+	$C_{15}H_{26}O$	222.37
	Myrtenal	+	$C_{10}H_{14}O$	150.22
	Geranyl acetone	+	$C_{13}H_{22}O$	194.31
	alpha-Cadinol	+	$C_{15}H_{26}O$	222.37
	Pinocarvone	+	$C_{10}H_{14}O$	150.22
Rhinacanthus nasutus	alpha-lapachone	+	$C_{15}H_{14}O_3$	242.27
	rhinacanthin-D	+	$C_{23}H_{20}O_7$	408.40
	Vanillic acid	+	$C_8H_8O_4$	168.15
	p-Hydroxybenzaldehyde	+	$C_7H_6O_2$	122.12

+ = positive result, - = negative result

Table 3: List of potential bioactive compounds exhibiting anti-inflammatory activity

Compound name	Molinspiration
3 o methyl quercitin	+
acetylaleuritolic acid	-
alpha –mangostin	-
Atractylenolide I	+
betulinic acid	-
Cacalol	+
Cacalone	+
caffeic acid	+
chlorogenic acid	-
Columbin	+
Costunolide	+
Cyanopicrin	+
Daphnodorin A	-
Daphnodorin b	-
Daucosterol	–
dehydrocostus lactone	+
Diphylline	+
eriodictyol	+
Febleucin	+
gamma mangostin	-
Geniposide	-
Germacrene D	-
Goniotholamin	+
Hispidulin	+
Hyperoside	-
Isocolumbin	+
isopentenyl guanidine	+
Jaseocidin	+
kaempferol -3-alpha	-
Lycopodine	+
Mansonone D	+
Monocrotaline	+
phyllamyricin B	+
phyllamyricin E	+
piper 1	+
Piperovatine	+
Supinine	+

+ = positive result, - = negative result

Table 4: Bioactive compounds which has cleared both AMES test and mutagenicity test

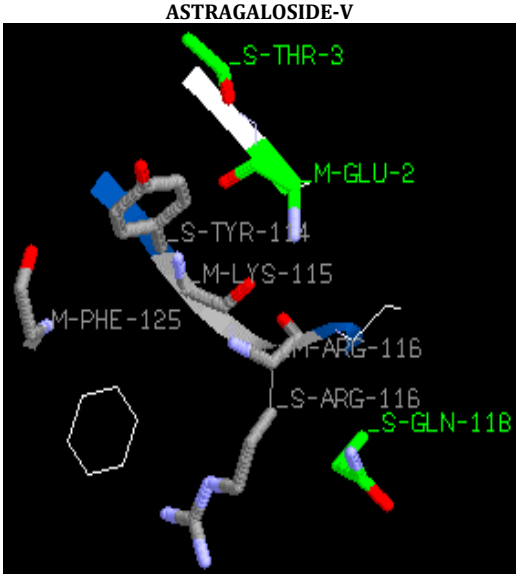
Compounds	Ames test	Mutagenicity
Astragaloside –II	+	+
Astragaloside –III	+	+
Astragaloside –III(0923)	+	+
Astragaloside –V	+	+
Astragaloside –IV	+	+
Astragaloside –VII	+	+
p-Hydroxybenzaldehyde	+	+
alpha –mangostin	+	+
Cacalone	+	+
Chlorogenic acid	+	+
Columbin	+	+
Daphnodorin A	+	+
Daphnodorin B	+	+
Daucosterol	+	+
Febleucin	+	+
Geniposide	+	+
Hyperoside	+	+
Isocolumbin	+	+
Kaempferol-3-α	+	+
Monocrotalline	+	+
Piperovatine	+	+

+ = positive result, - = negative result

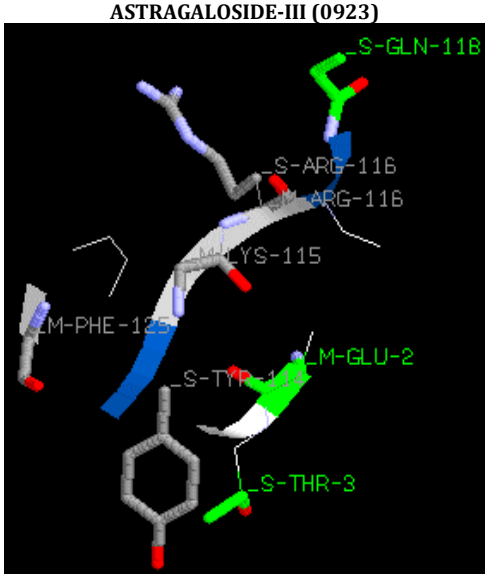
Table 5: Best docking score of bioactive compounds against DNA polymerase processivity factor along with its interaction value and hydrogen bench stretch

Compounds	Total Energy	Vanderwaals bonds	Hydrogen bonds
ASTRAGALOSIDE-V	-149.06	-123.48	-25.57
ASTRAGALOSIDE-III (0923)	-136.85	-127.35	-9.5
ASTRAGALOSIDE-IV	-130.33	-108.07	-22.26
ASTRAGALOSIDE-III	-129.22	-120.83	-8.4
ASTRAGALOSIDE-II	-125.44	-118.82	-6.62
ASTRAGALOSIDE-VII	-105.59	-85.31	-20.28
p-Hydroxybenzaldehyde	-97.44	-84.88	-12.96
Daphnodorin A	-78.74	-69.41	-9.33
Daphnodorin B	-78.11	-66.78	-11.34
Hyperoside	-72.46	-52.76	-19.7
kaempferol -3-alpha	-72.46	-52.76	-19.7
Daucosterol	-70.88	-60.74	-10.14
Monocrotaline	-65.73	-51.05	-14.68
Geniposide	-65.72	-51.45	-3.5
chlorogenic acid	-60.62	-47.9	-12.72
ISOCOLUMBIN	-58.49	-52.5	-5.99
COLUMBIN	-58.18	-52.18	-6
Cyanopicrin	-58.11	-52.38	-5.73
alpha –mangostin	-56.67	-50.05	-6.62
Febleucin	-56.54	-47.19	-9.35
Cacalone	-53.46	-46.46	-7
Piperovatine	-46.2	-40.2	-6

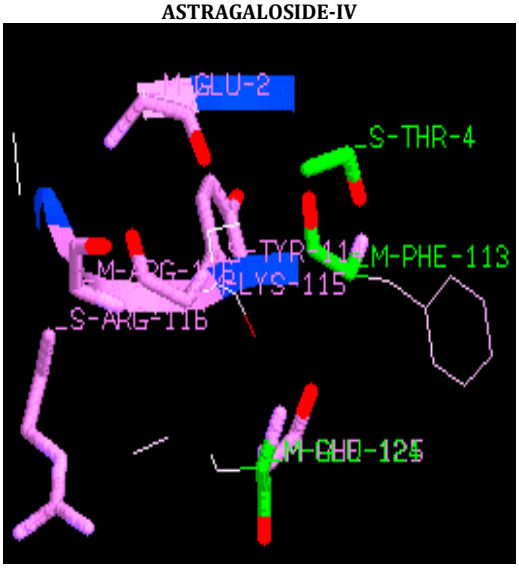
ASTRAGALOSIDE-V



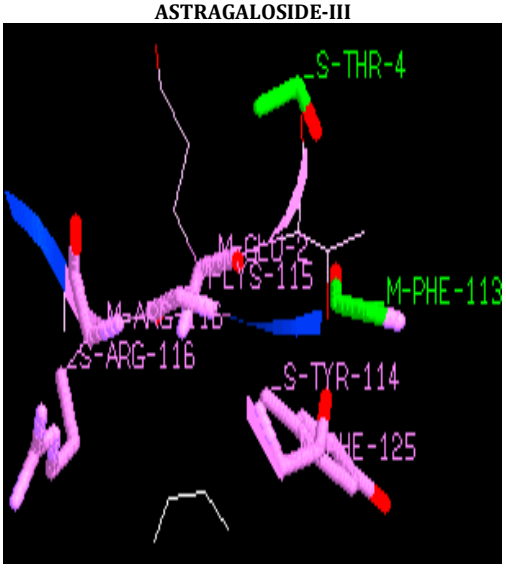
ASTRAGALOSIDE-III (0923)



ASTRAGALOSIDE-IV



ASTRAGALOSIDE-III



ASTRAGALOSIDE-II

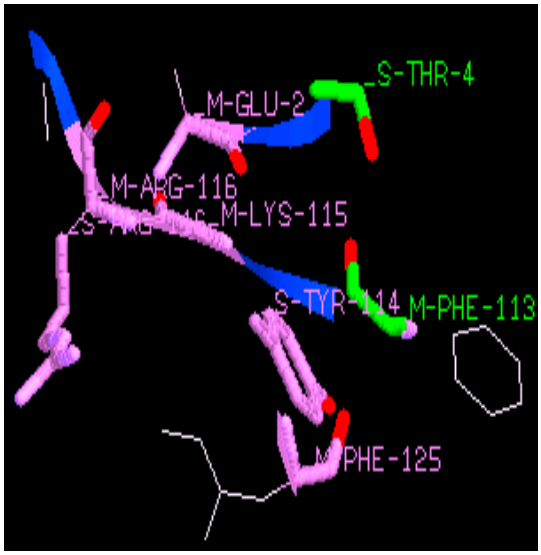


Fig. 1: Best interaction between BMRF1 and bioactive compounds

Table 6: Residue involved in hydrogen bond interaction with compound 1-5

	Compound 1	2	3	4	5
Glu-2	✓	✓	x	x	x
Thr- 3	✓	✓	x	x	x
Thr- 4	x	x	✓	✓	✓
Tyr-114	x	x	x	x	x
Lys-115	x	x	x	x	x
Arg-116	x	x	x	x	x
Gln-118	✓	✓	x	x	x
Phe-113	x	x	✓	✓	✓
Glu-124	x	x	✓	x	x

Table 7: Residue involved in Vanderwaals bonds interaction with compound 1-5

	Compounds 1	2	3	4	5
Glu -2	x	x	✓	✓	✓
Thr-3	x	x	x	x	x
Lys-115	✓	✓	✓	✓	✓
Arg-116	✓	✓	✓	✓	✓
Pro-117	x	x	x	x	x
Tyr-114	x	✓	✓	✓	✓
Phe125	✓	✓	x	✓	✓

Astralogoside V belonged to the family of Astragalus membranaceus exhibiting antioxidant properties. Depending on the anatomical part of the root, Astragaloside was differently concentrated and is the most predominant bioactive compounds extracted from the root of Astragalus membranaceus. The most Astragaloside-rich part of the root of A.membranceus was found to be the periderm [17]. The spectroscopic analysis was performed to elucidate the structure of Astragaloside I, acetylastragaloside I, Astragaloside I, Astragaloside III and Astragaloside IV after the compound were isolated from the hairy root cultures of Astragalus membranaceus [18]. A series of cycloartane triterpene glycosides represented Astragaloside I-VII was found in the root of the Astragalus. It had about one to three sugars attached at the 3-,6-, and 25-positions and found on the aglycone cycloastragenol [19]. The Astragalus facilitated the activation of T-cells, natural killer cells and also improved the production of immunoglobulin and macrophage [20]. Astragalus was found to be effective against systemic lupus erythematosis (SLE) [21], cancer, AIDS patients [22], *Shigella dysenteriae, Streptococcus hemolyticus, Diplococcus pneumonia,* and *Staphylococcus aureus* [23], leucopenia, human papillomavirus type 16 (HPV-16), *Herpes simplex* virus type 2 (HSV-2), and cytomegalovirus (CMV) [24], cardiovascular disease [25], genitouninary and renal disorders [26].

Using computational molecular docking, three new sulfated small molecules belonging to xanthones and flavonoids families were screened against EBV in Burkitt lymphoma. These molecules were identified by *in silico* analysis proved to effective against EBV when tested on Raji cell line [27].

It is well established from the previous studies that A.membranceus exhibited antiviral and antibacterial activity against many infections. From the present study, Astralogoside V belonging to A.membranceus was effective against BMRF1 gene, DNA polymerase processivity factor of Epstein barr vius.

CONCLUSION

Effective interaction between protein-ligand make the basis for structure based drug design. Till date, no antiviral drug or vaccine is available for this virus. Here we have attempted to identify inhibitors against BMRF1, DNA polymerase processivity factor. It

was found that if Astragaloside-V and Astragaloside-III showed the best interaction value against BMRF1 protein.

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