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A Computational approach to Identify Potential and Safer Antivirals targeting the E1 Protein of Chikungunya Virus

S. Rajarajan*

Bioinformatics infrastructure Facility centre of DBT, PG&Research dept of Microbiology& Biotechnology, Presidency college, Chepauk, Chennai-05. INDIA.

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ABSTRACT

Chikungunya virus (CHIKV), a positive stranded alphavirus, causes epidemic febrile infections characterized by severe and prolonged arthralgia. A few emerging strains had caused complications like neuralgia and ocular infections during recent infection. At present there is no specific drug available to inhibit the virus and are treated symptomatically with antipyretic drug & anti-inflammatory drugs. Hence the present study was to identify potential compounds derived from plant sources targeting E1 protein of Chikungunya virus. The structure of E1 protein was elucidated by homology modelling using PHYRE2. About 100 compounds with lower molecular weight were screened from the library and out of it 27 were selected based on Lipinski rule. The compounds that showed the best docking score using Molegro virtual docker and iGemdock were further screened for the toxicity assessment using PreADMET. Isopentenyl guanidine, jaseocidine, Phyllamyricin B, piperine, Dehydrocostus lactone showed the best docking score .Toxicity analysis by PreADMET had revealed that piperine and Phyllamyricin B was non carcinogenic to rats and mice. Also both compounds exhibited high plasma binding protein and blood brain barrier efficiency. Hence both Piperine and Phyllamyricin B could be potential drug candidates for inhibition of Chikungunya virus.

Key words: *Chikungunya virus, E1 protein, PHYRE 2, Molegro virtual docker PreADMET.*

INTRODUCTION

Chikungunya virus has emerged as an major human arbovirus pathogen since 2005 causing outbreaks in central, southern, west Africa, India and the islands of southern Indian Ocean, Indonesia, Malaysia (Sergon *et al*. 2005; Nimmannitya *et al*. 1969; Kariuki Njenga *et al.* 2008; Sang *et al.* 2005; Borgherini *et al*. 2007; Dash *et al.* 2007; Retuya *et al.* 1998; Sissoko *et al.* 2008; Ermould *et al.* 2008; Mavalankar *et al.* 2008; Beesoon *et al.* 2006; Sergon *et al.* 2004).

Also a few imported infections were reported from France, Italy ,Europe ,Australia (Chretien *et al.* 2007; Angelini *et al.* 2007; Rezza *et al.* 2007; Lanciotti *et al.* 2006; Panning *et al*. 2008).

The genome of alphaviruses is a single RNA molecule of positive polarity, about 11.5 kb in length, encoding four non structural proteins (nsp1-4) that are the essential components of the viral replicase and transcriptase .Functional proteins are first expressed as a polyprotein that is the cleaved by the viral proteases. Chikungunya virus contains three structural proteins: glycosylated E1 and E2 embedded in the viral envelope and a nonglycosylated nucleocapsid protein . (Renault *et al.* 2007; Simizu *et al.* 1984; Afjal Hossain Khan *et al.* 2002; Sourisseau *et al.* 2007).

E1 glycoprotein is 439 aminoacids long and the glycosylated part is conserved. E1 glycoprotein, a class II fusion protein is involved in the fusion process with host cell membrane. A conformational change occurs in the viral envelope proteins due to the low pH which results in the dissociation of the E2-E1 heterodimers, and formation of E1 homotrimers (Schuffenecker 2006; Inamadar *et al.* 2008).

Chikungunya virus infection has recently been reported to cause varied ocular manifestations like non granulomatous anterior uveitis, episcleritis, panuveitis, granulomatous anterior uveitis, optic neuritis, sixth nerve palsy, retrobulbar neuritis, retinitis with vitritis, neuroretinitis, keratitis, central retinal artery occlusion, choroiditis, exudative retinal detachment and secondary glaucoma,unilateral papillitis, bilateral papillitis, retrobulbar neuritis, perineuritis, neuroretinitis. (Inamadar 2008; Mittal 2007; Rose 2011).

In addition, during the Indian Ocean CHIKV Outbreak, a small proportion of the patients (about 123 out of 2, 44,000 infected) developed severe clinical signs such as neurological signs and hepatitis (Schuffenecker 2006).

Patients are treated with analgesics and anti-inflammatory agents based on the symptoms. Although chloroquine was reported to inhibit CHIKV virus *in vitro*, a double bind placebo-controlled randomised trial failed to show detectable antiviral effect. Also chloroquine resistant mutants were obtained by growing CHIKV in increasing concentrations of quinine (Rajan Ravichandran and Manju Manian, 2008; Lamballerie et al., 2008; Brighton 1984; Briolant 2004). Ribavirin is able to inhibit CHIKV but the expensive and parenteral injection is found to be unsuitable to treat the patients in large scale during epidemics (Briolant 2004). Except a few compounds like apigenin, naringenin, sylibin & glychrrhizic acid, a wide range of natural compounds remains to be tested (illenia Delougu *et al.* 2011; Kaur Parveen *et al.* 2012; Pohjala *et al.* 2012; Simona Ozden 2008).

The unavailability of specific antivirals for the treatment and a licensed vaccine to prevent the infection of Chikungunya virus necessitates the search for natural plant compounds having antichikungunya activity using bioinformatics tools and software. Henceforth Virtual screening would help to reduce the unnecessary evaluation of large number of compounds and time. Also the use of PREADMET test could facilitate in pruning the compounds for toxicity to the cell lines and will eliminate the compounds with mutagenic and carcinogenic activity (Jinn-Moon Yang *et al.* 2007; Subhomoi Borkotoky 2012; Abhik Seal *et al.* 2011).

Hence the natural compounds known to have potential pharmacological properties with low molecular weight were selected from the library to study the antichikungunya activity especially targeting the outer envelope E1 protein that could block the viral entry.

***Corresponding author:**

S. Rajarajan

Bioinformatics infrastructure Facility centre of DBT, PG&Research dept of Microbiology& Biotechnology, Presidency college,Chepauk, Chennai-05. INDIA.

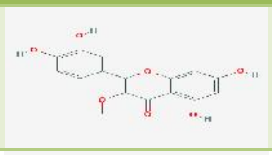
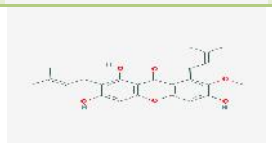
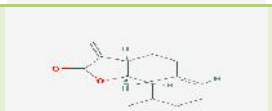
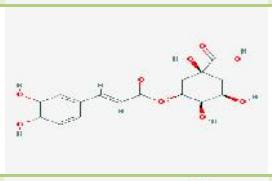
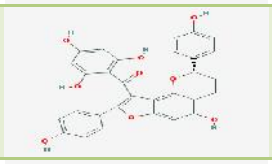
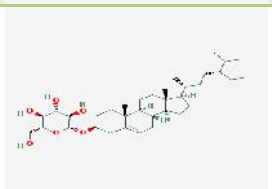
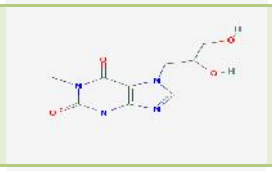
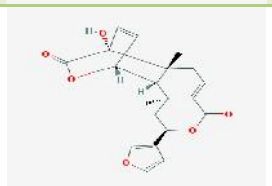
**E-Mail: drsrajarajan@gmail.com*

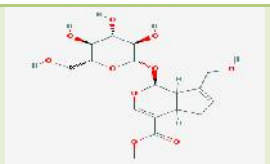
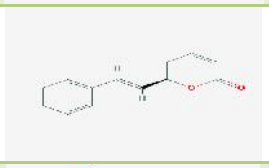
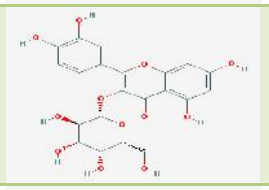
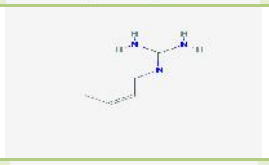
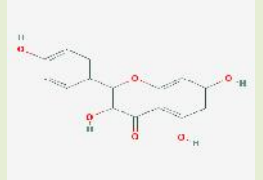
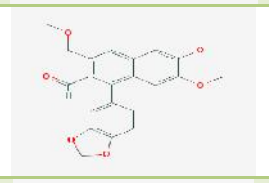
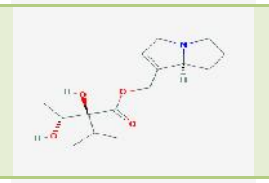
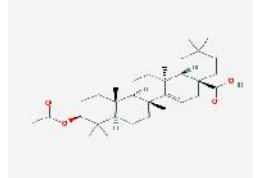
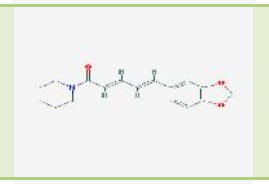
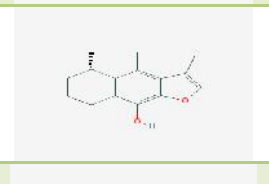
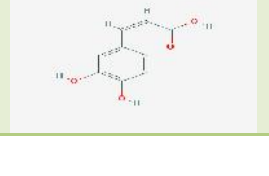
MATERIALS AND METHODS

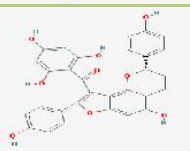
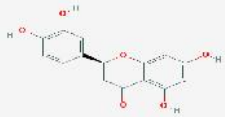
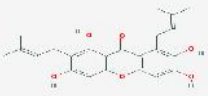
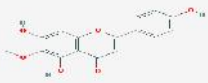
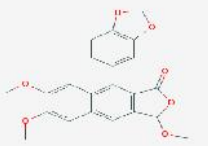
Ligand selection and SMART screening:

The ligand for E1 protein was retrieved from PubChem Compound database. The ligand structure, name, molecular formulas are given in **Table 1**. The ligand compounds were of molecular weight between 200 to 550 .The ligands were downloaded as XML file format from PubChem compound. The XML files were converted into the PDB 3D structure using marvin sketch.

Table No. 1: Potential plant compounds selected for docking studies with their chemical structure, Molecular formula and Molecular weight

S. No.	Compound	Structure	Molecular formula	Molecualr weight
1	3 o-methyl quercitin		$C_{16}H_{12}O_7$	316.26228
2	Betulinic acid		$C_{30}H_{48}O_3$	456.70032
3	Cacalone		$C_{15}H_{18}O_3$	246.30162
4	Alpha-mangostin		$C_{24}H_{26}O_6$	410.45964
5	Costunolide		$C_{15}H_{20}O_2$	232.3181
6	Chlorogenic acid		$C_{16}H_{18}O_9$	354.30872
7	Daphnadorin A		$C_{30}H_{22}O_9$	526.49028
8	Daucosterol		$C_{35}H_{60}O_6$	576.8473
9	Dyphylline		$C_{10}H_{14}N_4O_4$	254.24256
10	Fibleucin		$C_{20}H_{20}O_6$	356.3692

11	Geniposide		$C_{17}H_{24}O_{10}$	388.36646
12	Goniotholamin		$C_{13}H_{12}O_2$	200.23318
13	Hyperoside		$C_{21}H_{20}O_{12}$	464.3763
15	Isopentenyl guanidine		$C_6H_{13}N_3$	127.18752
16	Kaemferol		$C_{15}H_{10}O_6$	286.2363
17	PhyllamyricinB		$C_{22}H_{20}O_6$	380.3906
18	Supinine		$C_{15}H_{25}NO_4$	283.3633
19	Acetylaleuritolic acid		$C_{32}H_{50}O_4$	498.737
20	Piperine		$C_{17}H_{19}NO_3$	285.33766
21	Cacalol		$C_{15}H_{18}O_2$	230.30222
22	Cis Caffeic acid		$C_9H_8O_4$	180.15742

23	Coulmbin		$C_{20}H_{22}O_6$	358.38508
24	Cyanopurin		$C_{13}H_9N_7O$	279.25686
25	Daphnodorin		$C_{30}H_{22}O_9$	526.49028
26	Dehydrocostus lactone		$C_{15}H_{18}O_2$	230.30222
27	Eriodictyol		$C_{15}H_{12}O_6$	288.25218
28	Gamma-Mangostin		$C_{23}H_{24}O_6$	396.43306
29	Germacrene D		$C_{15}H_{24}$	204.35106
30	Hispidulin		$C_{16}H_{12}O_6$	300.26288
31	Isocolumbin		$C_{20}H_{22}O_6$	358.38508
32	Lycopodine		$C_{16}H_{25}NO$	247.3758
33	Phyllamyricin E		$C_{22}H_{18}O_7$	394.37412

Key physicochemical properties of compounds such as Molecular weight, ClogP, TPSA, Heavy atoms, HBA (Hydrogen Bond Acceptor), HBD (Hydrogen Bond donor), volume and rotatable bond were considered in this analysis for the preliminary screening of compounds that could exhibit physicochemical properties for favourable absorption, distribution, metabolism, excretion and toxicological parameters to be a suitable drug using online software molinspiration server.

Modelling of E1 protein of Chikungunya and SAVS:

The three-dimensional structure of E1 protein of Chikungunya virus was predicted using PHYRE2 (**Fig. 1**). The techniques of Homology Modelling, Secondary Structure Prediction and Domain analysis were used in the process. (**Table 2**)

Table No. 2: Protein identification and source

S.No.	Name	Description
1.	Accession Number	NP_690589
2.	Uniprot ID	Q8JUX5
3.	Protein Name	E1 envelope glycoprotein
4.	Host	*Aedes aegypti*
5.	Organism	Chikungunya virus (S27-African prototype)
6	Query protein	Q8JUX5
7	PDB structure model similar to the query Protein	C3j0cG ,3n42F ,c2yewB, c2xfbF ,c2xfcD, c2alaA, c3muuA, c3muwA, c1Id4O

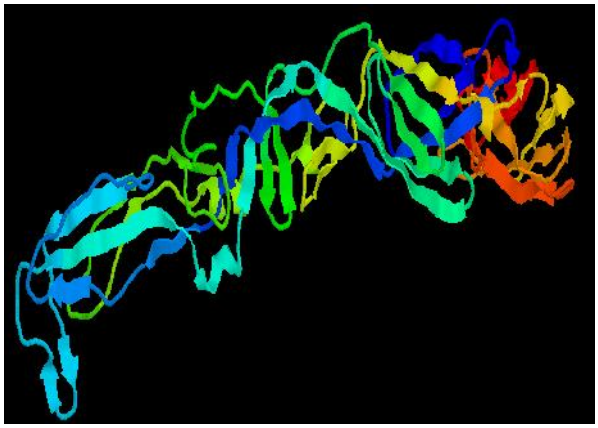


Fig. 1: Three dimentional Modelling of E1 protein

Structural Analysis and Verification Server (SAVS) was used for validating the 3D structure of protein and Ramachandran plot generated by SAVS were used for validating the given 3D structure of protein. E1 protein structure was elucidated and checked using ERRAT2 and PROCHECK (**Fig. 2**).

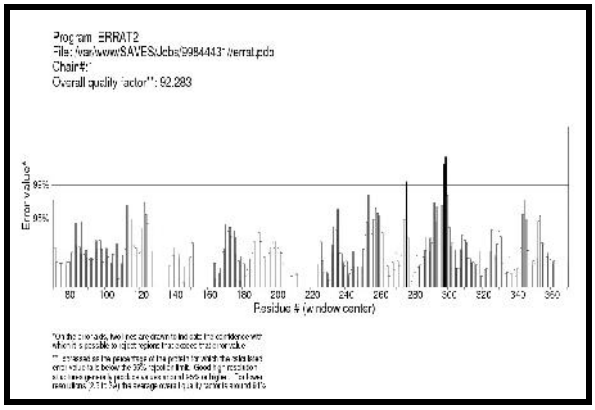


Fig. 2: Validation of protein using ERRAT$_2$

Docking analysis and Evaluation:

Determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligands was performed using Molegro Virtual Docker. Docking was performed to predict both ligand orientation and binding affinity using Molegro virtual Docker. Also the orientation of drugs in particular target was predicted. Knowledge of the preferred orientation in turn was used to predict the strength of association/binding affinity between two molecules using scoring functions. Docking score was further used to rank the Ligands on the basis of their relative binding affinities. The results were evaluated by analyzing docked drugs based on their scores. The docking scoring functions, E score, the ligand – protein interaction energy is given as

$$E_{inter} = E_{PLP}(r_{ij})\, 332.0\, q_i q_j / 4r_{ij}^2$$

i= ligan; j= protein; PLP= piecewise linear potential;

The summation runs over all heavy atoms in the ligand and all heavy atoms in the protein including any cofactor atoms and water molecule atoms that might be present. *EPLP* is a "piecewise linear potential" using two different sets of parameters for the steric (Van der Waals) term between atoms and hydrogen bonds.

ADMET:

PreADMET were used for assessing the disposition and potential toxicity of a ligand within an organism. Human Intestinal Absorption (HIA) and skin permeability model was predicted so that oral delivery and transdermal delivery was identified by using drug based models for *in vitro* Caco2-cell and MDCK cell assay. Also (BBB) blood brain barrier penetration of therapeutic drug in the central nervous system (CNS) and plasma protein binding model in its disposition and efficacy was identified using PreADMET. Simultaneously, mutagenicity by Ames TA 100, Ames TA 1535, Ames TA 98 and Carcinogenecity in rats and mouse were detected.

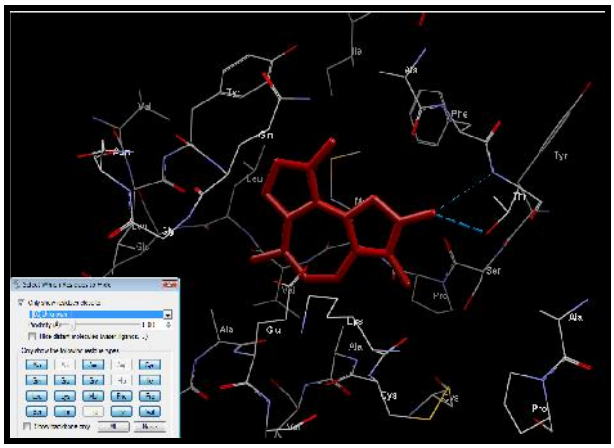
RESULTS

Smart screening filtered 25 compounds amongst the 100 selected compounds from library based on the drug likeliness properties such as Adsorption, Distribution, Metabolism, and Excretion (ADME). (**Table 1**). Isopentenyl guanidine, jaseocidine, Phyllamyricin B, Phyllamyricin E, Piperine, Dehydrocostus lactone showed the best docking score for E1 protein of Chikungunya virus as predicted by Molegro virtual docker (**Table 3**).

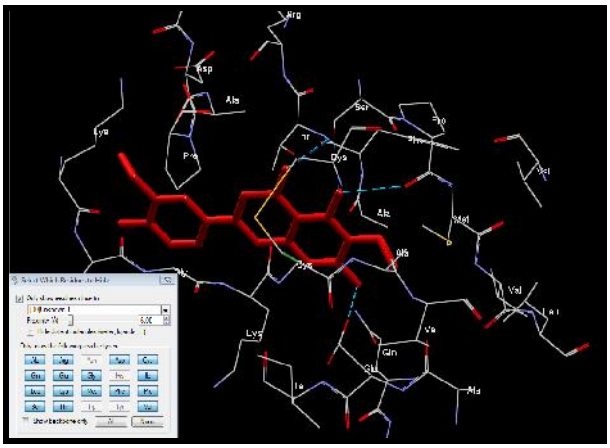
Table No. 3: Compounds with the best docking score, interaction value, hydrogen bend stretch and the aminoacids involved in interaction

Compounds	Docking score	Interaction	H bond	Amino acid interaction
Piperine	-124.688	-123.632	-5.36296	Ala,,Asn,Arg,Asp,Cys,Gln,Glu,Gly,Ile,Leu,Lys,Met,Phe,Pro, Ser,Thr,Tyr,Val
Phyllamyricin E	-117.438	-111.755	-2.15036	Ala,,Asn,Arg,Asp,Cys,Gln,Glu Gly,,Lys,Met,Phe,Pro,Ser,Thr,Tyr,Val
Dehydrocaluslactone	-112.426	-98.4709	-0.553097	Ala,Asn,Cys,Gln,Glu,Gly,Ile,Leu,Lys,Met,Phe,Pro ,Ser,Thr,Tyr,Val
Phyllamyricin b	-112.562	-116.051	-7.5	Ala,,Asn,Asp,Cys,Gln,Glu,Gly,His,Ile,Leu,Lys,Met ,Phe,Pro,Ser,Thr,Val
Jaseocidin	-95.9858	-96.3438	-4.58707	Ala,Arg,Asp,Cys,Gln,Glu,Gly,Ile,Leu,Lys,Met,Phe ,Pro,Ser,Thr,Val
Isopentenylguanidine	-77.5833	-75.1123	-0.818141	Ala,Asn,Gln,Glu,Gly,Ile,Leu,Lys,Met,Phe,Tyr, Val

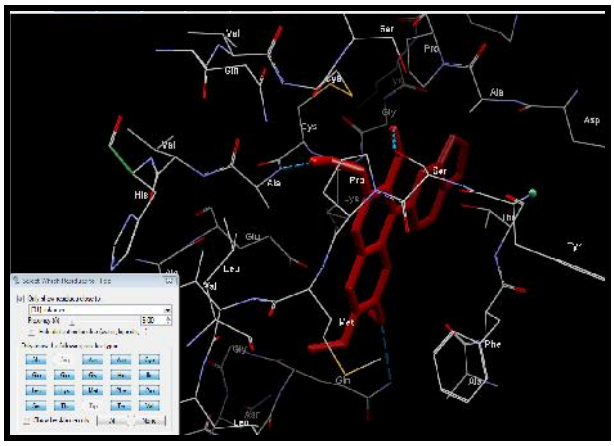
Although a wide range of amino acids in the active site of E1 protein of CHIKV interacted with these four top lead molecules ,hydrogen bond interaction occurred between the Ser, Gln, Leu, Gly residues of E 1 protein of ChikV and Piperine; Phyllamyricin B interacted by stretching out the hydrogen bond with Ala, Glu, Gly & Glu, Ser residues of E 1 protein of ChikV interacted with Phyllamyricin E. Dehydrocostus lactone were able to interact with Ala, Asn, Cys, Gln, Glu, Gly, Val. (**Fig. 3 & 4**).



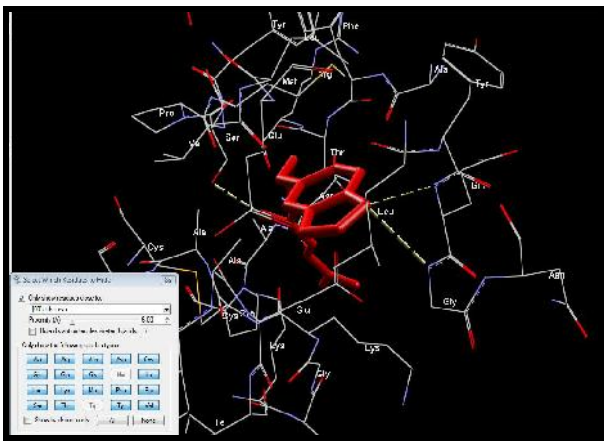
3.1). Dehydrocaluslactone



3.2). Jaseocidin

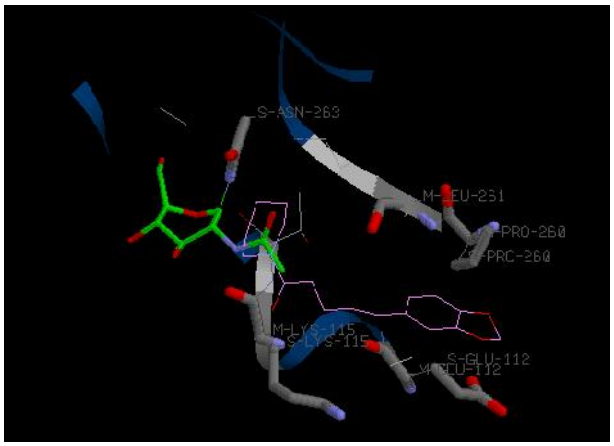


3.3). Phyllamyricin B

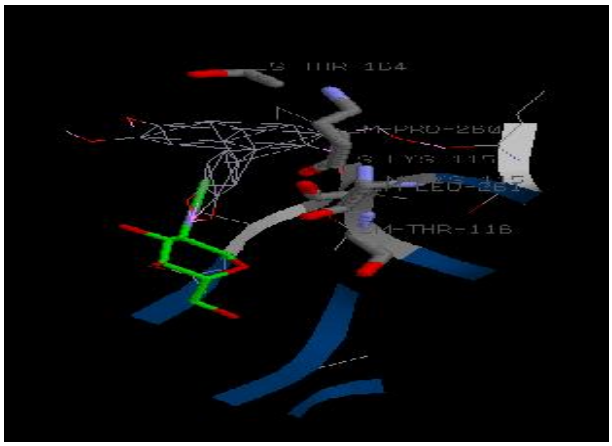


3.4). Piperine

Fig. 3: Docking conformations and hydrogen bonding of 3.1) Dehydrocaluslactone, 3.2) Jaseocidin 3.3) Phyllamyricin B 3.4) Piperine to the best binding cavity site of Chikungunya E1 protein. Atoms of E1 protein are shown in white lines and the Compounds (ligands) are shown in CPK model (red colour). Hydrogen bond interaction of the ligand with the protein is stretched by blue dashed lines.Also the aminoacids involved in the interaction is shown in the left side of the table.



4.1). Piperine



4.2). Phyllamyricin B

Fig. 4: Interaction of Piperine and Phyllamyricin B with the aminoacid residues in Envelope protein of Chikungunya virus Thr and Jaseocidin with the aminoacids Ser, Phen, Meth, Glu.

PreADMET further screened the lead compounds based on the mutagencity and carcinogenicity tests. PreADMET test showed the mutagenecity profile by Ames TA 100, Ames TA 1535, Ames TA 98 in the presence and the absence of S-9 liver cells. Piperine and Jaseocidin were mutagenic to the Ames TA 100 in the presence of S-9 Liver cells thus signifying the chances of base pair

substitution parallely the compounds Isopentenylguanidine, Phyllamyricin B were mutagenic to Ames TA 98 in the presence of S-9 liver cells and hence the likely concern of frameshift mutation . Although that the above mentioned compounds were mutagenic to liver cells they are found to be non carcinogenic in both rats and mouse. LIPINSKI rule selected Phyllamyricin E and Dehydrocostuslactone as a druglikeliness compound however Phyllamyricin E and Dehydrocostuslactone were found to be both mutagenic and carcinogenic as predicted by toxicity testing in PreADMET and hence suggesting the unsuitability of Phyllamyricin E and Dehydrocostuslactone as a drug for human use.

Phyllamyricin b and piperine had shown high intestinal absorption value of 95.895 & 90.65 % respectively in comparison to Isopentenylguanidine (66%) and Jaseocidin (60%). The plasma binding efficiency and also the blood brain barrier penetration was higher for Phyllamyricin B and piperine than Isopentenylguanidine and Jaseocidin.

DISCUSSION

Owing to the unavailability of an effective antiviral to treat the chikungunya infection, search for new antivirals from the available plant compounds with known potential pharmacological properties with the help of bioinformatics tools would help reduce the time and cost. Screening of plant compounds by virtual Molegro Docker, PreADMET helped in the identification of two lead molecules Piperine and Phyllamyricin B.

In this study a well developed docking tool Molegro virtual docker was utilised to perform virtual screening on the selected plant compounds from pubchme database that could dock the identified active cavity of E1 Protein. The possible interaction of these compounds on the non-structural protein (nsp2) other than the structural protein (E1 protein) was ruled out and confirmed by docking analysis. None of the compounds mentioned in **Table 3** docked neither showed the interaction with viral helicase and protease protein which has a prime role in the replication of viral RNA and hence proving the potential role of piperine and phyllamyricin B as an entry inhibitor to Chikungunya virus.

Although ,the compounds phyllamyricin B and piperine exhibited interaction by showing the difference in aminoacid residues, a strong hydrogen-bonding network provided strong attraction forces that stabilized the binding differences in their interaction. Compared to the stability of the E 1 protein, only the prefusion assay of docking was attempted and also the unnecessity screening of small molecules and chemical substances were avoided due to cellular toxicity of the substances.

In addition, PreADMET helped to assess the toxicity profile equivalent to that of *in vitro* testing of mutagenicity and carcinogenicity. Phyllamyricin B and piperine were non carcinogenic and found safer. Moreover, the high intestinal absorption value suggested the oral administration of drug than the parenteral mode of drug delivery.

Even though Jaseocidin, Dehydrocaluslactone, isopentenyguanidine, Phyllayricin E showed strong interaction by forming hydrogen bonds between the aminoacids and E 1 Protein, their carcinogenecity and mutagenecity made them as unlikely candidates as drugs.

Piperine, an alkaloid known to possess various pharmacological activities such as antidepressant activity, cognitive enhancing effect, bioenhancer properties, inhibition of apoptosis, antitumor activity, antioxidant, antiplatelet effect, anti-inflammatory activity, hepatoprotective effect, antithyroid effect, antiasthmatic activity and antileishmanial activity. Phyllamyricin B (1-(1,3-benzodioxol-5-yl)-6,7-dimethoxy-3-(methoxymethyl)naphthalene-2-carbaldehyde) has been shown to exert antiviral activity.

Henceforth the supportive *in vitro* and *in vivo* analysis on the antiviral activity of Piperine and Phyllamyricin B to Chikungunya virus in the near future could definitely prove Piperine and Phyllamyricin B as an promising antiviral candidates for the treatment of Chikungunya virus.

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