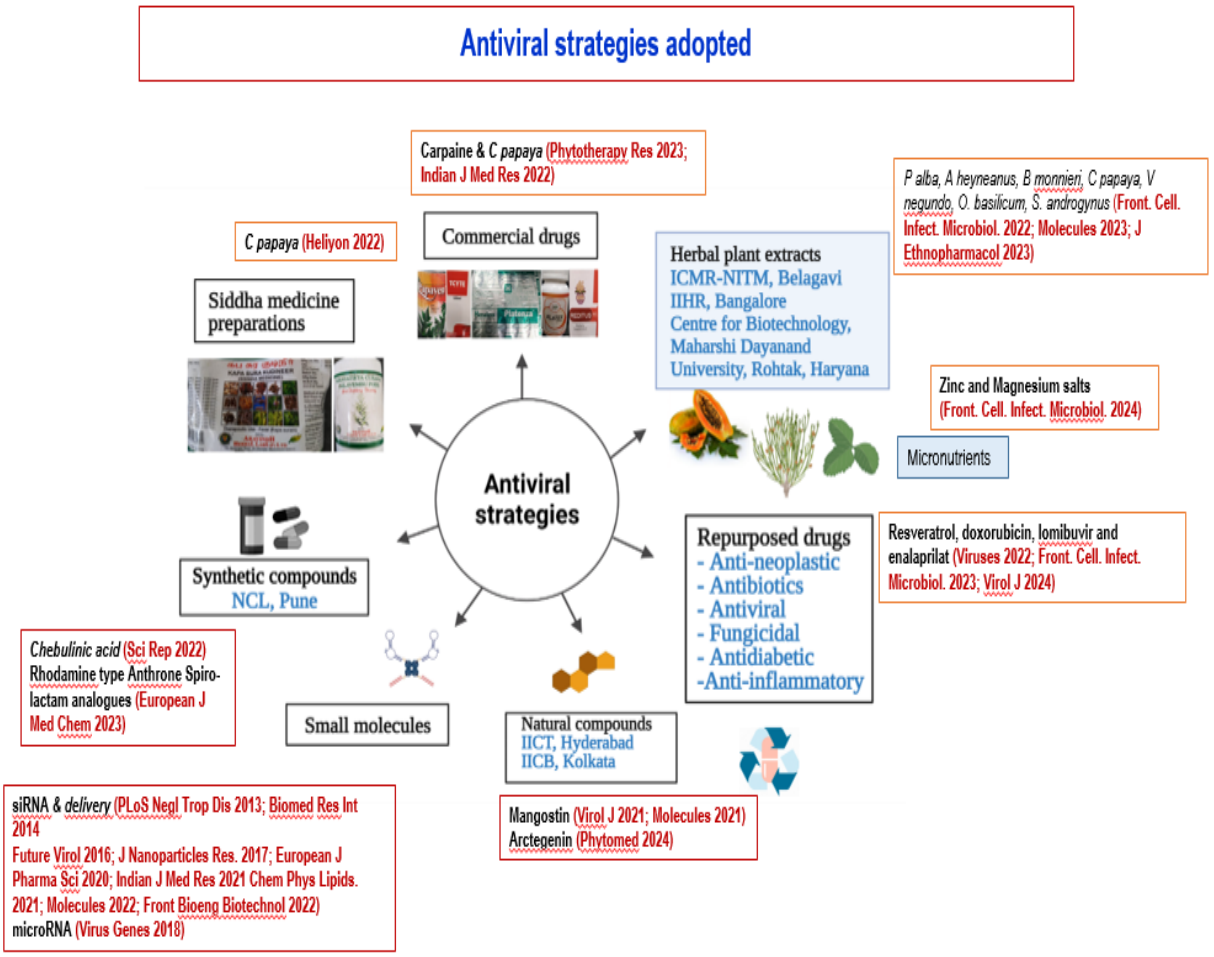
Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Dengue and chikungunya virus infections are important causes of morbidity and mortality in tropical and subtropical parts of the world. Dengue virus (DENV) and chikungunya virus (CHIKV) are transmitted through *Aedes aegypti* and *Aedes albopictus* mosquitoes. Both of the viruses cause acute febrile illness, and symptoms wise, both diseases are identical in the acute phase, though the clinical presentation differs as the infection progresses. There are no licensed antivirals/vaccines available against DENV and CHIKV, and their prevention is still based on vector control measures. Therefore, the need for effective drugs with anti-dengue and anti-chikungunya activities is imperative ***(Parashar et al 2014)***.
In the area of antiviral therapeutics, I have worked on RNA interference, natural & synthetic compounds, traditional medicinal plant extracts and repurposed drugs. The anti-dengue and anti CHIKV activity studies using *in vitro*/ *in vivo* assays is described below:



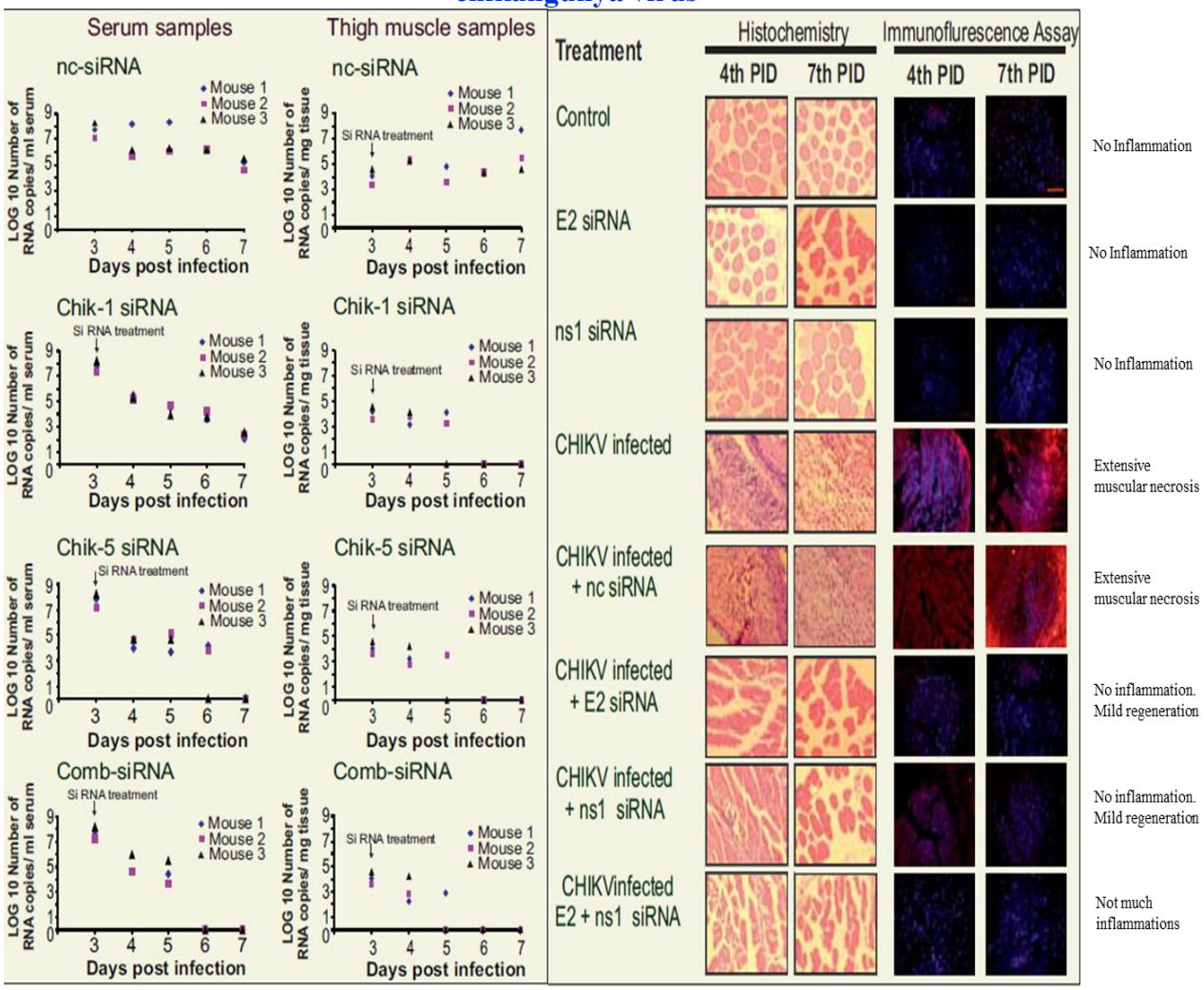
RNA interference: Inhibition of virus replication and gene expression by directly introducing siRNAs into the cells have been reported for several viruses ***(Parashar & Cherian 2014, Parashar & Cherian 2016, Panda et al 2021)***.

RNAi agent for inhibition of Chikungunya virus

Pioneering work done in the area of RNA interference agent (siRNA) for the inhibition of chikungunya virus which was published ***(Parashar et al, 2013; patents granted in US (2017), China (2019) Europe (2019), Australia (2021) & India (2021).***

The efficacy of the siRNAs against ns1 and E2 genes of CHIKV both *in vitro* and *in vivo* have been evaluated. Four siRNAs each, targeting the E2 and ns1 genes were designed and evaluated for efficiency in inhibiting CHIKV growth *in vitro* and *in vivo*. Two siRNAs were effective in controlling CHIKV replication in vitro as assessed by different assays. CHIKV replication was completely inhibited in the virus-infected mice when administered 72 hours post infection (h.p.i.). The combination of two siRNAs exhibited additive effect leading to early and complete inhibition of virus replication. These findings suggest that RNAi capable of inhibiting CHIKV growth might constitute a new therapeutic strategy for controlling CHIKV infection and transmission.

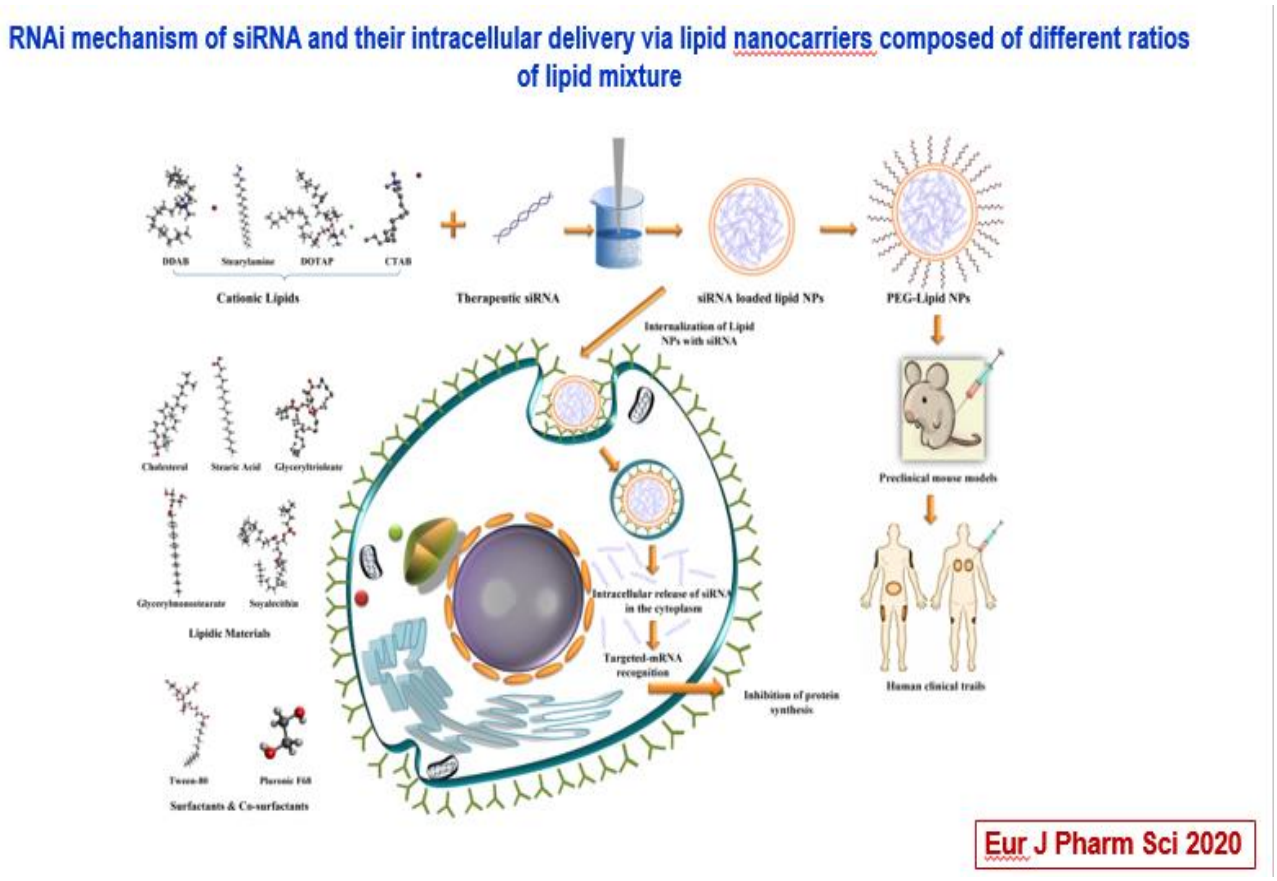
Inhibition of CHIKV replication after transfection with siRNA in mice infected with chikungunya virus



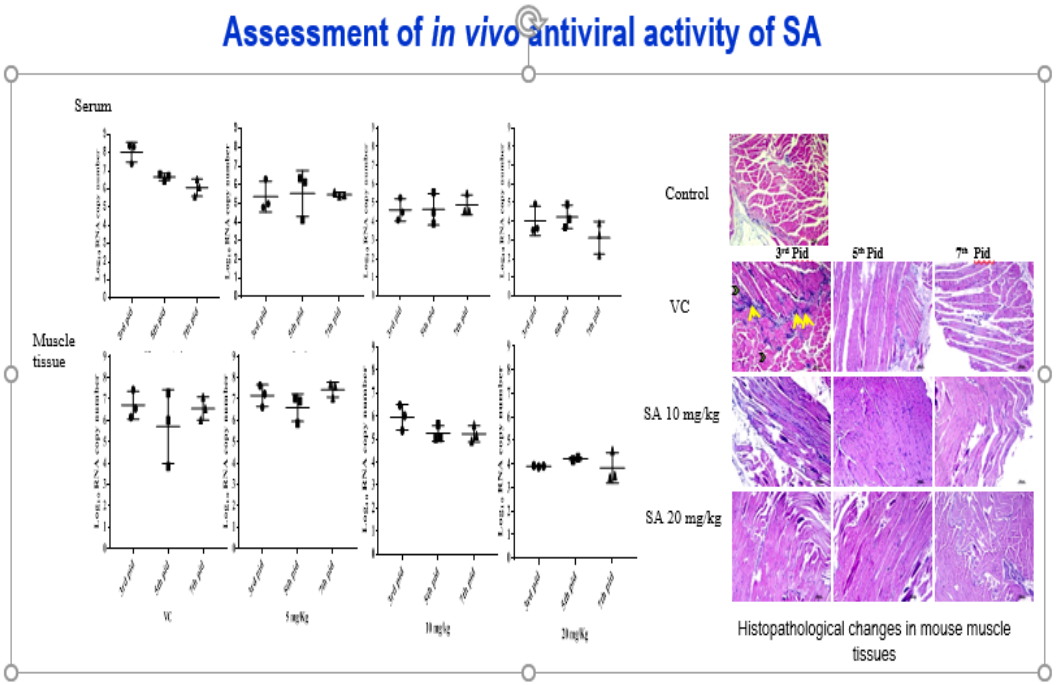
(PLoS Negl Trop Dis, 2013, Biomed Res Int 2014, Future Virol 2016)
Patent granted: US (2017), China (2019) Europe (2019), Australia (2021) & India (2021)

Development of delivery systems for targeted delivery of siRNAs for chikungunya treatment

The real therapeutic potential of siRNA is limited due to the fact that unprotected oligonucleotides such as - unmodified siRNA for ns1 and E2 genes have a very short half-life *in vivo* (seconds to minutes) due to degradation (by endogenous nucleases) and rapid kidney filtration from circulation (due to their small size). For these reasons, enhanced stability and site specific siRNA delivery strategies through employment of novel nanoparticle based delivery vehicles are promising because nanoparticles prevent degradation or rapid clearance, enhance uptake and promote endosomal escape of therapeutic siRNA ***(Parashar et al 2020)***. However, it is important that the nanoparticle vehicles for drug delivery must demonstrate siRNA binding, low cytotoxicity, effective cellular uptake, and, most importantly, evidence of siRNA-induced knockdown. Hence the attempt to develop therapeutic siRNA encapsulating solid lipid nanoparticles (SLN) & Zeolitic imidazolate framework for CHIKV antiviral therapy have been done.



Cationic lipids: For the first time we have reported the efficacy of Stearylamine (SA), a cationic lipid alone against CHIKV. Our findings demonstrate the potential of SA in effectively inhibiting the viral replication in infected Vero cells thereby decreasing the viral load in C57BL/6 mice. SA has a direct effect and helps ameliorate acute disease symptoms in CHIKV-infected mice. Promising results from this study frames a commensurate basis to develop a suitable delivery system along with a drug or gene against chikungunya as a novel antiviral therapy. This preclinical study provides evidence to support further studies to develop SA based formulations to treat chikungunya fever effectively ***(Jeengar et al 2021)***.

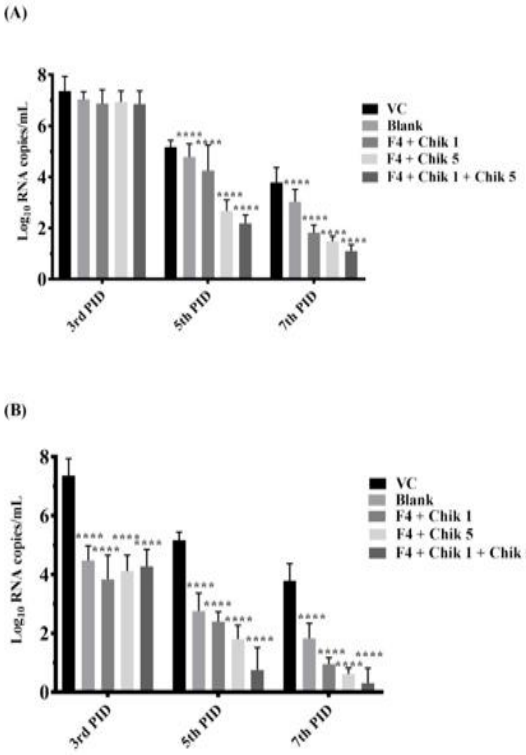


SA was used as cationic lipid and treatments in the C57BL/6 mice revealed that SA showed strong inhibition of CHIKV.

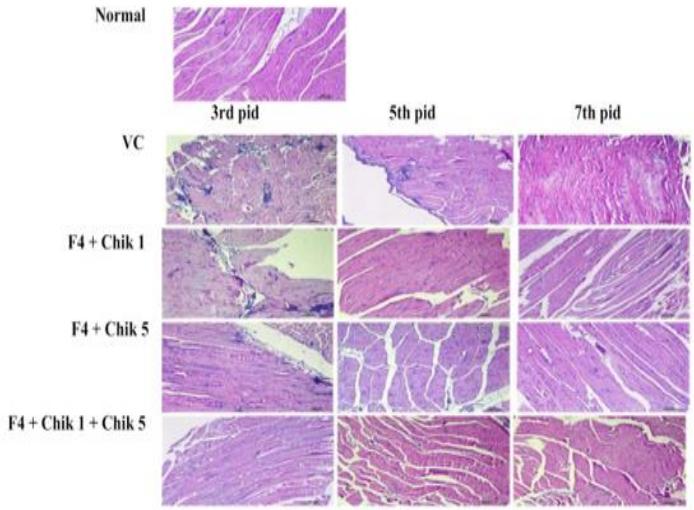
Chem Phys Lipids 2021

Soli Lipid Nanoparticles: Nanodelivery systems were prepared, characterized and complexed with siRNA targeting E2 and NS1 gene region. The developed four delivery systems (F1,F2,F3 and F4) were assessed for stability and potential toxicities against CHIKV. In comparison to the other nanodelivery systems, F4 having allowing maximum siRNA complexation, better stability and higher transfection with strong inhibition against E2 and NS1 genes of CHIKV. The study concludes that cationic lipid like stearylamine with ease of synthesis and characterization, indicated maximum complexation by structural condensation of siRNA owing high transfection alone and synergistic inhibition of CHIKV along with siRNA both *in-vitro* and *in-vivo* models. Therefore, stearylamine based cationic lipid nanoparticles can be embraced and explored as safe, potent and efficient no viral vectors overcoming siRNA *in-vivo* complexities against chikungunya. In future, nanoparticles containing siRNA approach can be used in developing delivery system for the treatment of other viral disease treatment ***(Jeengar et al 2022)***.

Effect of cationic lipid nanoparticle loaded siRNA with stearylamine against chikungunya Virus



In vivo anti-CHIKV activity of siRNA-complexed SLN delivery system. The reduction in CHIKV copies/mL in (**A**) serum and (**B**) muscle tissue after treatment with the siRNA-loaded F4 delivery system

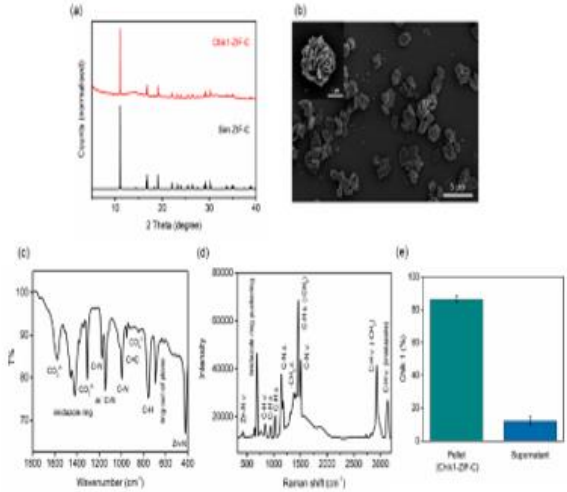


Histopathological changes in mouse muscle tissues after chikungunya infection and siRNA-loaded F4-delivery-system treatment

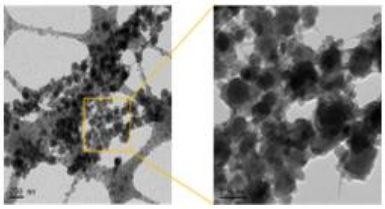
Molecules 2022

Zeolitic imidazolate framework: The therapeutic efficiency of siRNA can be improved by using an efficient delivery system. Metal-organic framework biocomposits have demonstrated an exceptional capability in protecting and efficiently delivering nucleic acids into cells. In the present study, carbonated ZIF called ZIF-C has been utilized to deliver siRNAs targeted against E2 and nsP1 genes of CHIKV to achieve a reduction in viral replication and infectivity. Cellular transfection studies of E2 and nsP1 genes targeting free siRNAs and ZIF-C encapsulated siRNAs in CHIKV infected Vero CCL-81 cells were performed. Our results reveal a significant reduction of infectious virus titre, viral RNA levels and percent of infected cells in cultures transfected with ZIF-C encapsulated siRNA compared to cells transfected with free siRNA. The results suggest that delivery of siRNA through ZIF-C enhances the antiviral activity of CHIKV E2 and nsP1 genes directed siRNAs ***(Tagore et al 2022).***

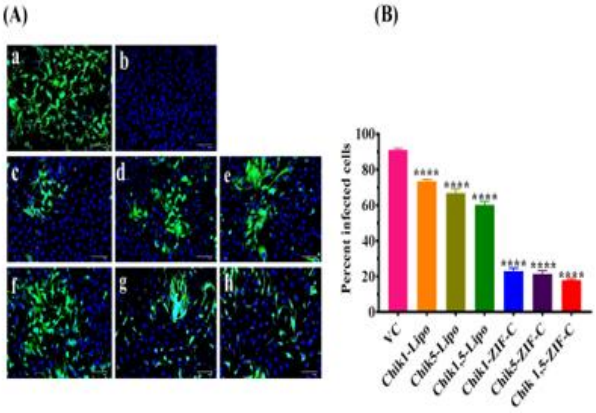
Targeted *in vitro* gene silencing of E2 and nsP1 genes of chikungunya virus by biocompatible zeolitic imidazolate framework



Characterization of Chik1-ZIF-C. (a) XRD of Chik1-ZIF-C was compared with simulated ZIF-C; (b) morphology was revealed by SEM; functional chemical groups and surface composition were studied by (c) FT-IR and (d) Raman spectroscopy; (e) quantification of encapsulated Chik1 sequence within ZIF-C MOF pellet.



TEM image of Chik1-ZIF-C



Effect of ZIF-C-siRNA formulations and lipofectamine mediated siRNA transfection on CHIKV infection. Immunofluorescent images (A) of Virus Control (a); Cell Control (b); Chik1-ZIF-C (c); Chik5-ZIF-C (d); Chik1,5-ZIF-C (e); Chik1-Lipo (f); Chik5-Lipo (g); Chik1,5-Lipo (h) at 0 h.p.i. Virus infected cells are stained green by FITC and the nucleus of the cells is stained blue by DAPI (a). Percent of infected cells during ZIF-C siRNA and Lipofectamine 2000 siRNA treatment (at 0 h.p.i). Cells were counted in four different fields to obtain the percentage of infected cells (B).

Front. Bioeng. Biotechnol 2022

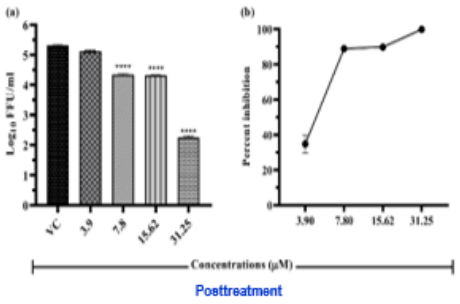
Traditional medicinal plant extracts and Natural compounds:

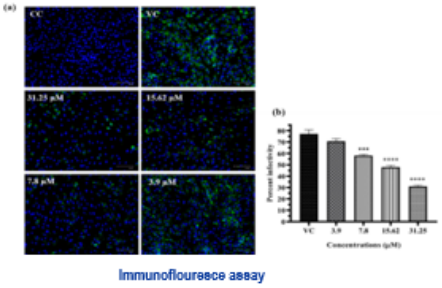
In vitro antiviral screening of 25 extracts prepared from the plants of *Vitex negundo, Plumeria alba, Ancistrocladus heyneanus, Bacopa monnieri, Anacardium occidentale, Cucurbita maxima, Simarouba glauca,* and *Embelia ribes* using different solvents and four purified compounds (anacardic acid, chloroquinone, glaucarubinone, and methyl gallate) were carried out for their anti-dengue virus (DENV) and anti-chikungunya virus (CHIKV) activities. Extracts from *Plumeria alba, Ancistrocladus heyneanus, Bacopa monnieri,* and *Cucurbita maxima* showed both anti-DENV and CHIKV activity while extract from *Vitex negundo* showed only anti-DENV activity. Among the purified compounds, anacardic acid, chloroquinone and methyl gallate showed anti-dengue activity while only methyl gallate had anti-chikungunya activity ***(Alagarasu et al 2022).***

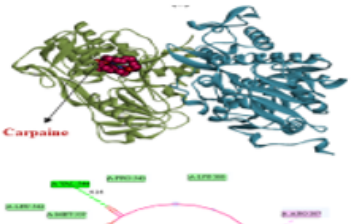
Carica papaya: The current study was undertaken to study the antiviral activity of commercially available *Carica papaya* leaves extract (CPLE) based products and CPLE prepared in four formulations against DENV and CHIKV. Maximum nontoxic concentrations of the commercially available CPLE based products and CPLE based formulations (*Carica papaya* leaves in powder form, *Carica papaya* leaves in lyophilized form, *Carica papaya* leaves based silver nanoparticles and supercritical fluid extract of *Carica papaya* leaves) were used for screening the antiviral activity. The results revealed *Carica papaya* leaves based silver nanoparticles and supercritical fluid extract of *Carica papaya* leaves formulations showed significant inhibition in case of DENV while papaya leaves in powder form showed significant reduction in case of CHIKV. This study demonstrates the antiviral activity of CPLE formulations against DENV and CHIKV infection in *in-vitro* system and needs further validation in *in-vivo* models ***(Patil et al 2022, Shrivastava et al 2022)***.

Carpaine*:** Traditional remedies are being used to treat dengue fever and many studies have reported the utilization of *Carica papaya* leaves extracts (CPLE) in treating dengue patients. CPLE have been extensively used to treat thrombocytopenia in several cases. Among the compounds reported from CPLE, carpaine is a major alkaloid and active compound. Carpaine has been reported to have antithrombocytopenic activity. Antiviral activity was observed under post-treatment conditions. The highest reduction was observed in infected cultures treated with 31.25µM of carpaine (from 5.318 in VC to 2.259 mean log10 FFU/ml). In infected cell cultures treated with 15.62 and 7.8 µM carpaine concentrations, one log reduction in virus titre was observed. Carpaine treatment led to a significant 2 log10 titre decrease in viral RNA copy number at 31.25 µM concentration compared to VC. In IFA, carpaine significantly decreased the percent of infected cells compared to VC. The findings suggest the anti-DENV property of carpaine post infection. The anti-DENV activity was confirmed by different assays which measure infectious virus titre, viral RNA copy number, and viral protein expression. The *in-silico* observation of stable binding of carpaine with NS5 RdRp suggests that carpaine might interfere with the functioning of viral replication. To conclude, the present study provides *in-vitro* and *in-silico* evidence of anti-DENV activity of carpaine against DENV-2 in Vero CCL-81 cells ***(Alagarasu et al 2023).

In vitro and *in silico* effect of carpaine, a major alkaloid of *Carica papaya* on dengue virus infection







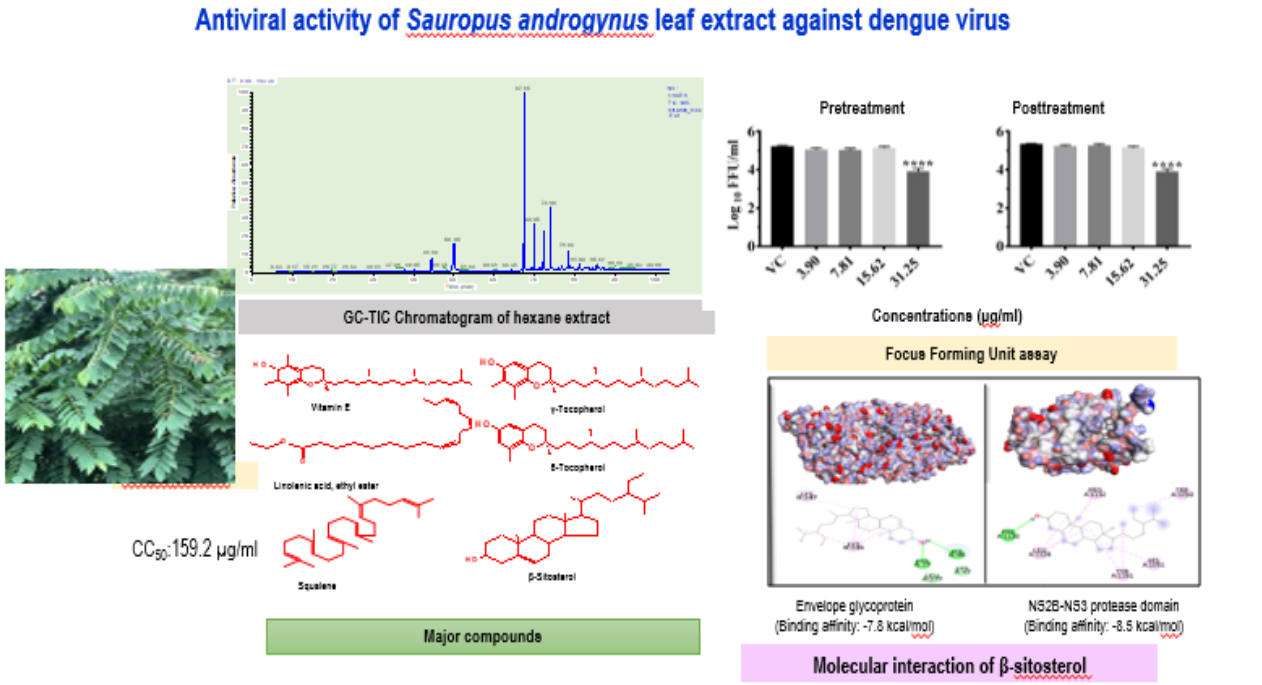
CC50 (µM)	EC50 (µM)	SI value
58.7	4.627	12.68

Name	Binding affinity (kcal/mol)	Residues	Bonding type
NS3 helicase domain	-10.1	Thr 224	Carbon-Hydrogen bond-1
		Arg 387	Alkyl bond-1
		Val 544	Hydrogen bond-1

- Carpaine possess antiviral activity against DENV replication and might have utility as a therapeutic agent against DENV infections.
- Docked to NS3 helicase domain, suggests that carpaine could interfere with the unwinding activity of the duplex viral RNA that is vital for viral replication.
- More evidence related to safety, antiviral and immunomodulatory activities need to be generated from animal models and clinical trials.

Phytotherapy Res 2022

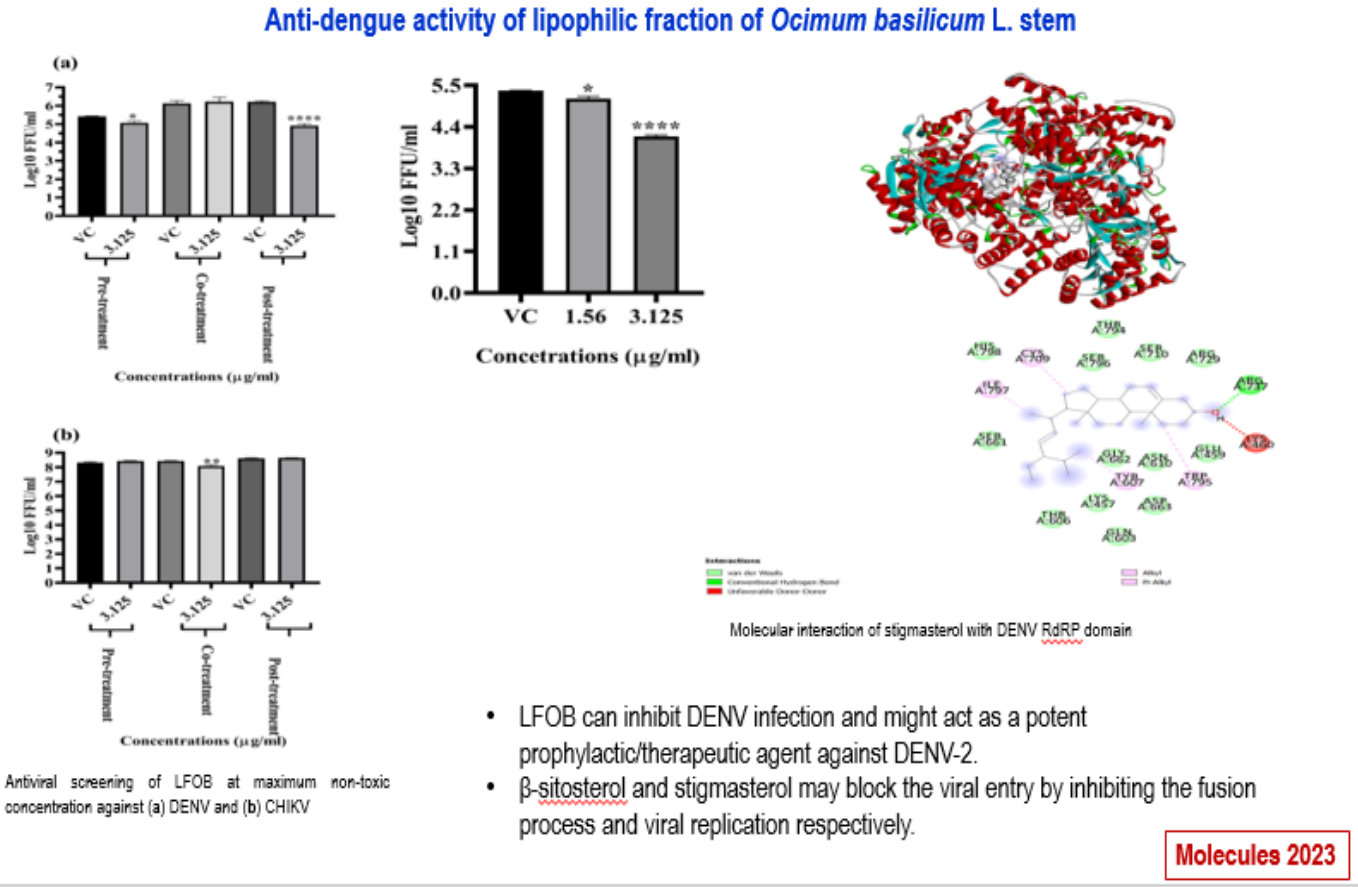
Sauropus androgynus**:* commonly known as "multigreen" and "multivitamin" is consumed as a vegetable and used in traditional medicine to relieve fever. This *in vitro* study is aimed to explore the activities of the lipophilic fraction of the leaves of *S. androgynus* (LFSA) against DENV, CHIKV and malaria parasite. Twelve compounds were identified in LFSA using GC/MS. The most abundant compound was squalene (36.9%), followed by vitamin E (12.5%) and linolenic acid (10.2%). Significant reduction in DENV titre was observed under pre- and post-infection treatment conditions at a concentration of 31.25 µg/ml, but no antimalarial and anti-CHIKV activity was observed. The Autodock-Vina-based in-silico docking study revealed that β-sitosterol could form a strong interaction with the DENV E glycoprotein. Our findings suggest that LFSA can inhibit DENV infection and might act as a potent prophylactic/ therapeutic agent against DENV. In-silico results suggested that β-sitosterol may block the viral entry by inhibiting the fusion process ***(Joshi et al 2023).



- Fraction of the leaves can inhibit DENV infection and might act as a potent prophylactic/therapeutic agent against DENV-2.
- *β*-sitosterol: interacts with the E protein of DENV and may block the viral entry or virus release by inhibiting the fusion process. Strong interaction with NS2B-NS3 protease domain might affect the virus protein cleavage and further function.

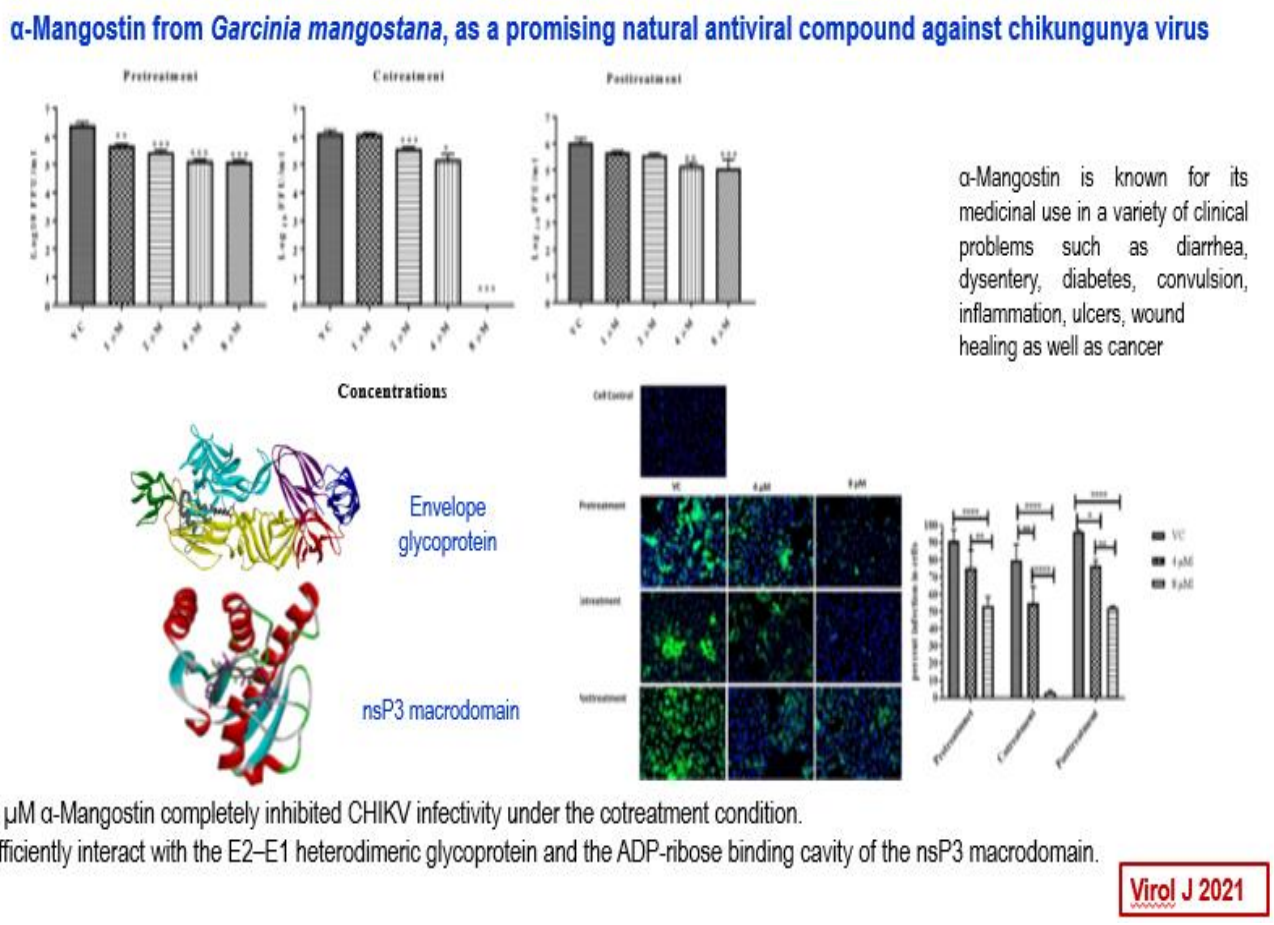
J Ethnopharmacol 2023

Ocimum basilicum: This plant is used to cure many types of fever in traditional medicine. This study aims to explore the antiviral activity of the lipophilic fraction of the stem of *O. basilicum* (LFOB) against DENV and CHIKV. The LFOB was analyzed using GC-FID and GC-MS. Twenty-six compounds were identified in LFOB using GC/MS. The most abundant compounds were β-sitosterol (22.9%), stigmasterol (18.7%), and campesterol (12.9%). Significant reduction in DENV titre was observed under pre- and post-infection treatment conditions at a concentration of 3.125 µg/mL, but no anti-CHIKV activity was observed. Our earlier and the present AutoDock-Vina-based *in silico* docking study revealed that β-sitosterol and stigmasterol could form strong interactions with the DENV E glycoprotein and DENV RdRp domain, respectively. Our findings suggest that LFOB can inhibit DENV infection and might act as a potent prophylactic/therapeutic agent against DENV-2. *In silico* results suggested that β-sitosterol and stigmasterol may block the viral entry by inhibiting the fusion process and viral replication respectively ***(Joshi et al 2023)***.

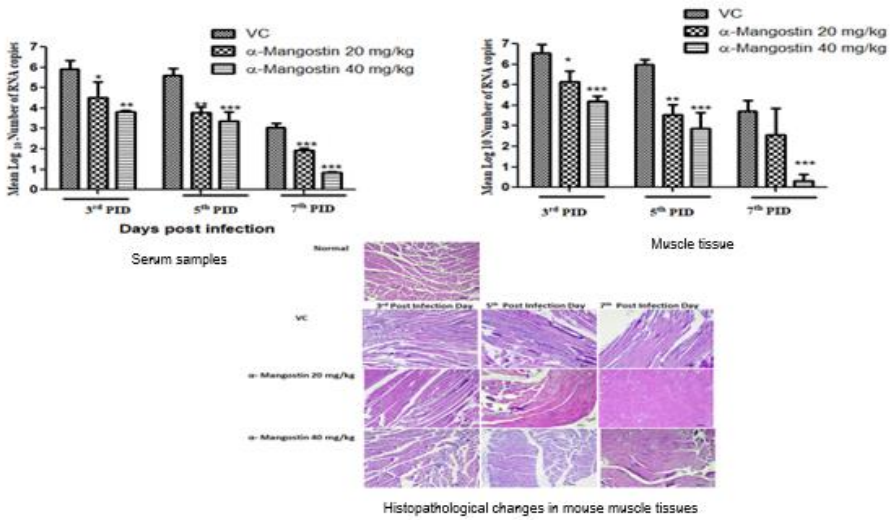


α-Mangostin: As diverse natural phenolic compounds have been shown to possess antiviral activities, we explored the antiviral activity of compounds, shortlisted using an *in-silico* near-neighbor search within the National MolBank repository of IICT, Hyderabad, against CHIKV both *in vitro* and *in vivo*.

In vitro studies revealed that α-Mangostin completely inhibited CHIKV infectivity under the cotreatment condition. CHIKV replication was also inhibited in virus-infected mice. This is the first *in vivo* study which clearly showed that α-Mangostin is effective *in vivo* by significantly reducing virus replication in serum and muscles. Molecular docking indicated that α-Mangostin can efficiently interact with the E2–E1 heterodimeric glycoprotein and the ADP-ribose binding cavity of the nsP3 macrodomain. The findings suggest that α-Mangostin can inhibit CHIKV infection and replication through possible interaction with multiple CHIKV target proteins and might act as a prophylactic/therapeutic agent against CHIKV ***(Patil et al 2021)***.



In vivo inhibition of chikungunya virus using α-Mangostin



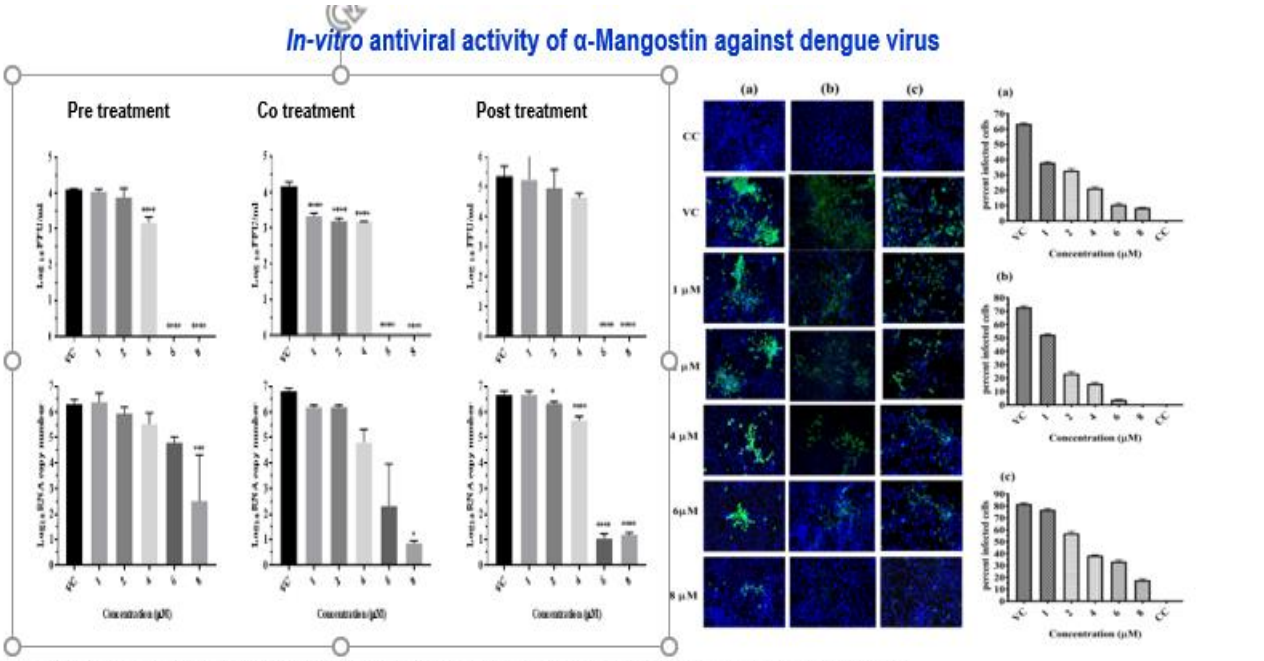
Histopathological changes in mouse muscle tissues

- First *in vivo* study showed that α-Mangostin is effective by significantly reducing CHIKV replication in serum and muscles

Virol J 2021

Natural compounds were also screened for their antiviral activity against DENV by *in vitro* cell line-based assay. α-Mangostin, a xanthanoid, was observed to exert antiviral activity against DENV-2 under pre-, co- and post-treatment testing conditions. The in vitro and in silico findings suggest that α-Mangostin possesses the ability to suppress DENV-2 production at different stages of its replication cycle and might act as a prophylactic/therapeutic agent against DENV-2 ***(Panda et al 2021)***.

In-vitro antiviral activity of α-Mangostin against dengue virus

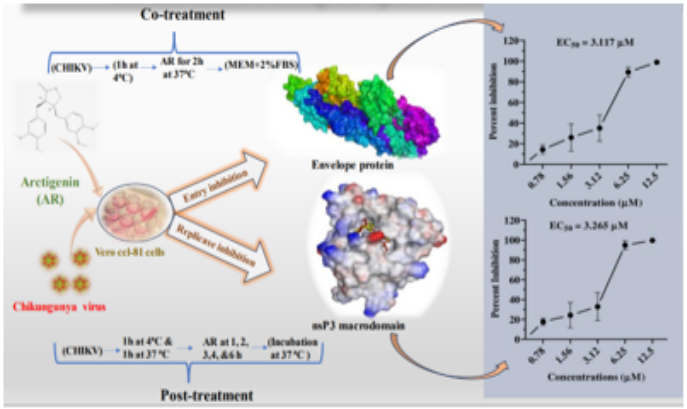


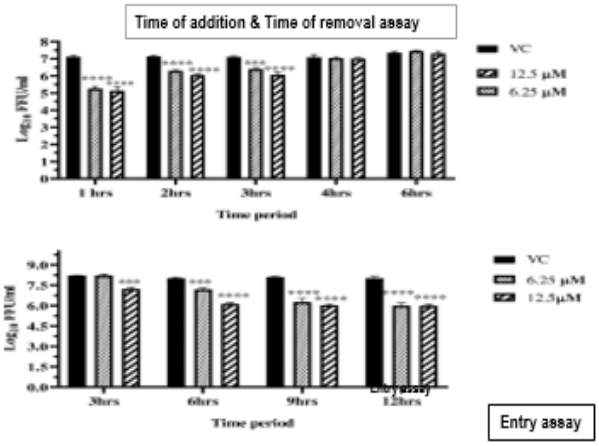
- 6 μM α-Mangostin completely inhibited DENV infectivity under all the *in vitro* treatment conditions.
- Mangostin can interact with multiple DENV protein targets such as the NS5 methyltransferase, NS2B-NS3 protease and the glycoprotein E.

Molecules 2021

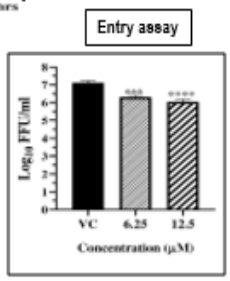
Arctigenin from Arctium lappa L. inhibits CHIKV by affecting its entry and replication: The antiviral activity of Arctigenin, a phenylpropanoid lignan from the seeds of *Arctium Lappa* L., have been investigated against dengue and chikungunya virus. Arctigenin had no effects on DENV. Various time and temperature dependent assays revealed that Arctigenin significantly reduced CHIKV RNA copy number and infectious virus particles in cell cultures. *In-silico* docking results revealed the interaction of the compound with E1 protein and nsp3 macrodomain of CHIKV. This study demonstrates the in-vitro anti-CHIKV potential of Arctigenin and suggests that the compound might affect CHIKV entry and replication ***(Shukla et al 2024)***.

Arctigenin from *Arctium lappa* L. inhibits CHIKV by affecting its entry and replication





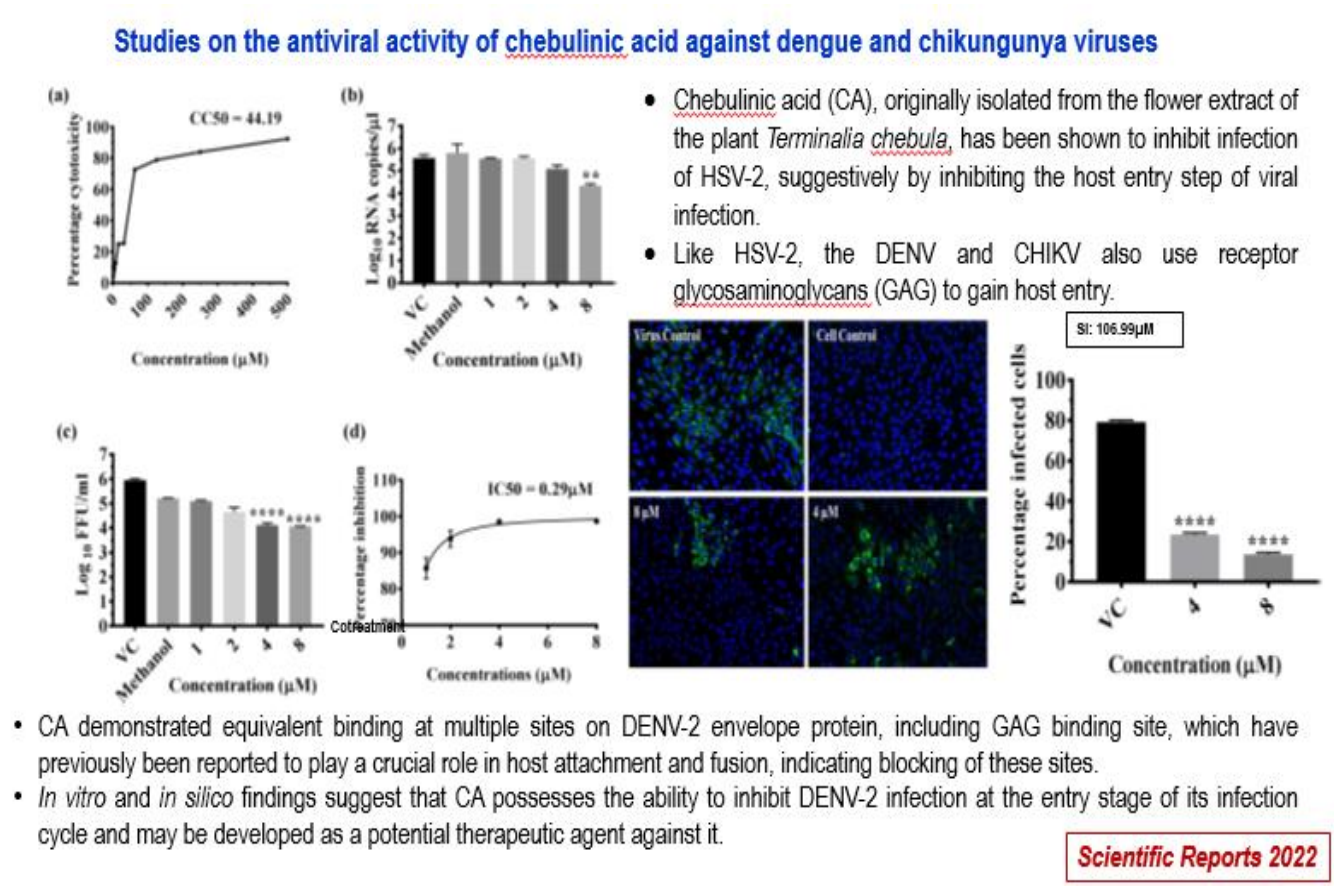
- **Attachment & Entry assay:** Arctigenin interferes with CHIKV entry
- **TOA & TOR:** a significant reduction in virus titre was observed (within 1-3 h after infection). Reduction increased with increased time of exposure to compound (Early treatment and long duration exposure of Arctigenin decreased viral load)
- **Bypass entry assays:** A significant decrease in infectious virus titre, negative strand RNA load and genomic RNA (inhibition in post entry stages)
- ***In silico:*** high binding affinity of the compound with the E1 protein and the nsp3 macrodomain



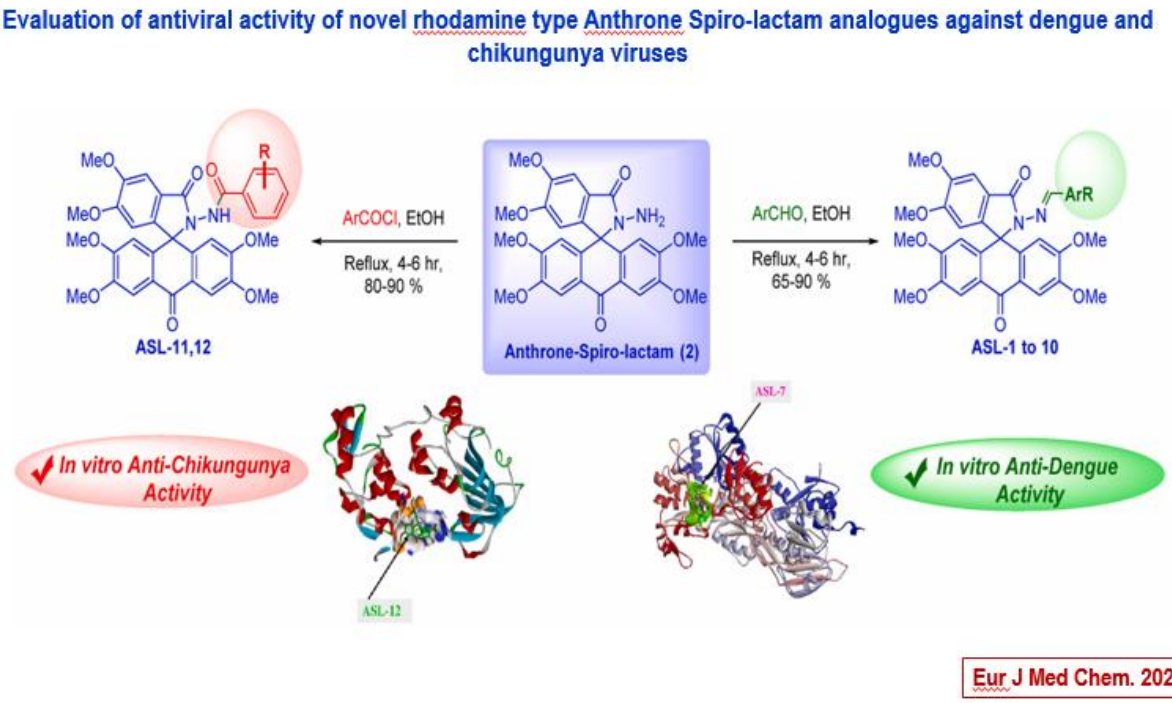
- **Study provides *in-vitro* and *in-silico* evidence of anti-CHIKV activity of Arctigenin in Vero CCL-81 cells and suggests that it can inhibit CHIKV by affecting viral entry and replication.**

Phytomedicine 2024

Chebulinic acid: This is originally isolated from the flower extract of the plant *Terminalia chebula*, has been shown to inhibit infection of herpes simplex virus-2 (HSV-2), suggestively by inhibiting the host entry step of viral infection. Like HSV-2, the DENV and CHIKV also use receptor glycosaminoglycans (GAG) to gain host entry, therefore, the activity of Chebulinic acid (CA) was tested against these viruses. Co-treatment of 8 µM CA with DENV caused 2 log decrease in the virus titer (4.0 log10FFU/mL) at 120 h post infection, compared to virus control (5.95 log10FFU/mL). In contrast, no inhibitory effect of CA was observed against CHIKV infection under any condition. The mechanism of action of CA was investigated in silico by employing DENV and CHIKV envelope glycoproteins. During docking, CA demonstrated equivalent binding at multiple sites on DENV envelope protein, including GAG binding site, which have previously been reported to play a crucial role in host attachment and fusion, indicating blocking of these sites. However, CA did not show binding to the GAG binding site on envelope protein-2 of CHIKV. The *in vitro* and *in silico* findings suggest that CA possesses the ability to inhibit DENV infection at the entry stage of its infection cycle and may be developed as a potential therapeutic agent against it ***(Thomas et al 2022)***.

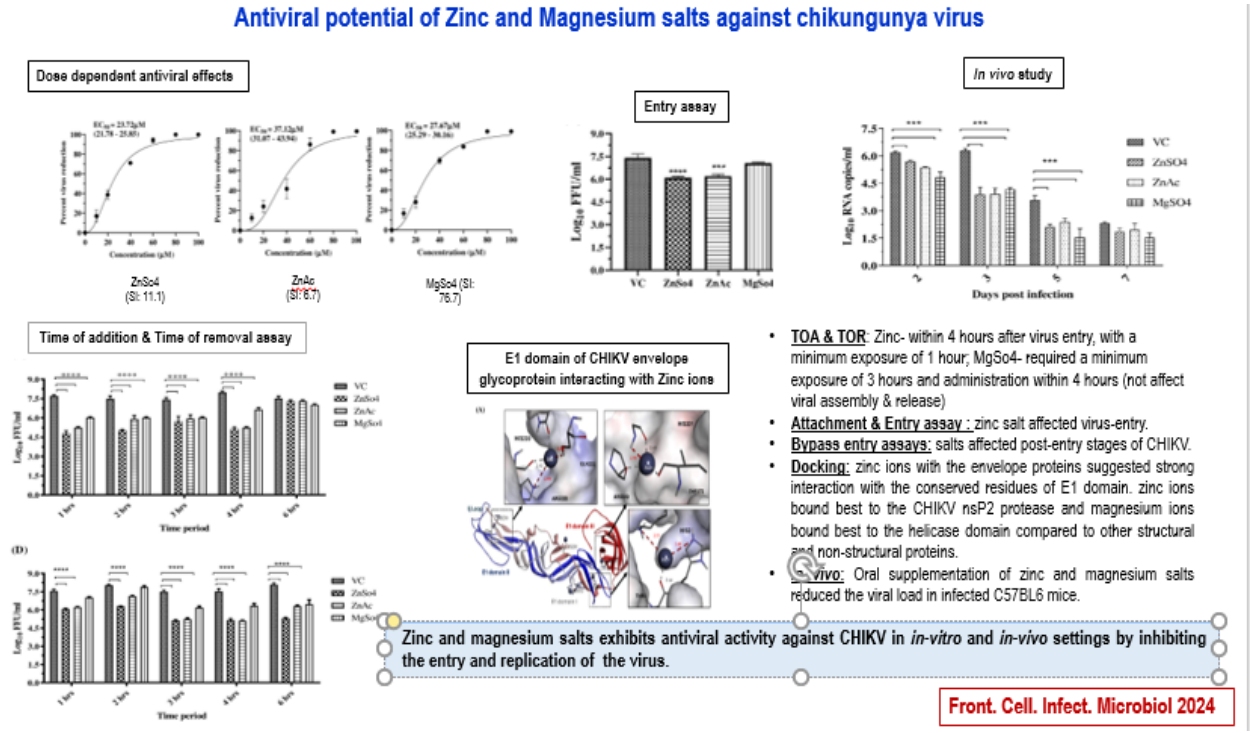


Novel rhodamine type Anthrone Spiro-lactam (ASL) analogues: A series of Rhodamine type Anthrone-Spirolactam (ASL) derivatives Benzylimin-Anthrone-Spirolactam (ASL-1 to ASL-10) and Benzamide-Anthrone-Spirolactam (ASL-11 and ASL-12) were synthesized via a simple condensation reaction between Anthrone Spiro-lactamine (2) and various aromatic aldehyde and acyl chlorides respectively by CSIR-NCL, Pune. Since rhodamine-based compounds were reported to have antiviral activity, the ASL derivatives were examined for *in vitro* antiviral activity against dengue and chikungunya viruses. Among all the analogues, ASL-3, ASL-6, ASL-7, ASL-8, ASL-9 and ASL-10 were the most potent against dengue virus (DENV) and exerted around one log reduction in virus titre under post-treatment conditions. At the same time ASL-3 was effective under cotreatment conditions. Two analogues ASL-6 and ASL-12 exerted anti-chikungunya virus (CHIKV) activity under post-treatment conditions. *In silico* docking studies revealed that the ASL derivatives interacted with the proteins of DENV and CHIKV. Together, the results suggest the anti-DENV and CHIKV activity of ASL derivatives which may be exploited further for therapeutic purposes.



Zinc and magnesium salts:

This study explored the antiviral potential of zinc sulphate, zinc acetate, and magnesium sulphate against CHIKV infection. Different time- and temperature- dependent assays revealed the therapeutic antiviral activity of zinc and magnesium salts against CHIKV. A minimum exposure of 4 hours and treatment initiation within 1 to 2 hours of infection are required for inhibition of CHIKV. Entry assays revealed that zinc salt affected virus-entry. Entry bypass assays suggested that both salts affected post entry stages of CHIKV. In infected C57BL6 mice orally fed with zinc and magnesium salts, a reduction in viral RNA copy number was observed. The study results suggest zinc salts exert anti-CHIKV activity at entry and post entry stages of the virus life cycle, while magnesium salt affect CHIKV at post entry stages. Overall, the study highlights the significant antiviral potential of zinc sulphate, zinc acetate, and magnesium sulphate against CHIKV, which can be exploited in designing potential therapeutic strategies for early treatment of chikungunya patients, thereby reducing the virus-associated persistent arthritis ***(Davuluri et al 2024).***

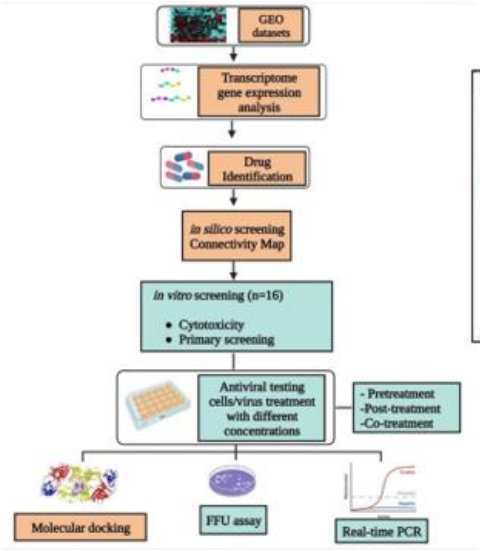


Repurposed drugs:

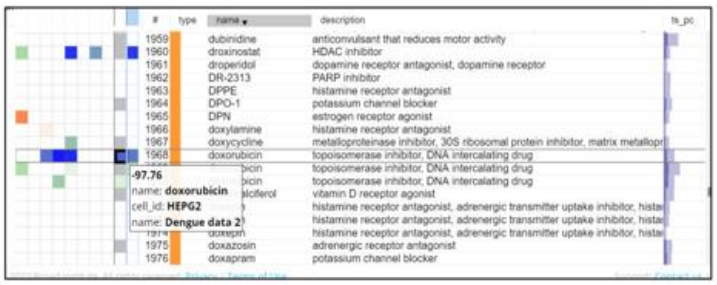
Among the many approaches employed to identify drugs to treat dengue and chikungunya, drug repurposing has gained popularity, which is safer and more economic. The targeted drugs have further been tested for their effectiveness against other diseases and proven safe for treatment of the human diseases. In recent years, drug repurposing has been applied in many studies to identify treatments.

A transcriptomics-based bioinformatics approach have been employed for drug identification against DENV. Gene expression omnibus datasets from patients with different grades of dengue disease severity and healthy controls were used to identify differentially expressed genes in dengue cases, which were then applied to the query tool of Connectivity Map to identify the inverse gene–disease–drug relationship. A total of sixteen identified drugs were investigated for their prophylactic, virucidal, and therapeutic effects against DENV. Focus-forming unit assay and quantitative RT-PCR were used to evaluate the antiviral activity. Results revealed that five compounds, viz., resveratrol, doxorubicin, lomibuvir, elvitegravir, and enalaprilat, have significant anti-DENV activity. Further, molecular docking studies showed that these drugs can interact with a variety of protein targets of DENV, including the glycoprotein, the NS5 RdRp, NS2B-NS3 protease, and NS5 methyltransferase The *in vitro* and *in silico* results, therefore, reveal that these drugs have the ability to decrease DENV-2 production, suggesting that these drugs or their derivatives could be attempted as therapeutic agents against DENV infections ***(Punekar et al., 2022).***

A transcriptomics-based bioinformatics approach for identification and *in vitro* screening of FDA-approved drugs for repurposing against dengue virus



In silico and *in vitro* methods used for selecting and studying the antiviral activity of drugs for repurposing



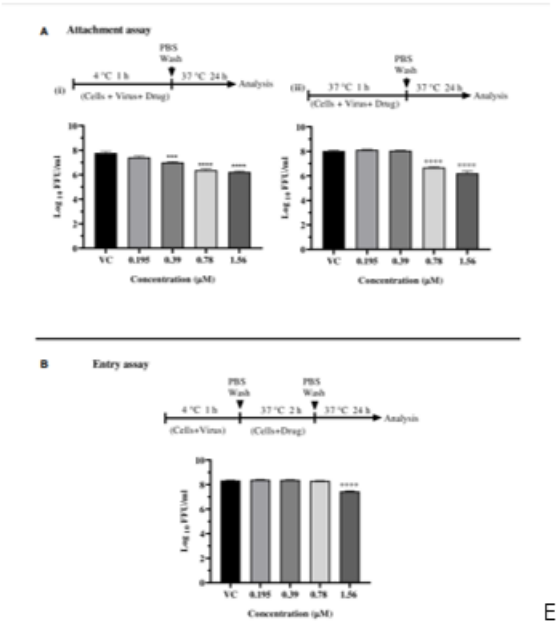
Snapshot of the heat map generated by querying the DHF DEGs in CMap. The blue coloured gradient indicates the negative connectivity score of the compounds with inverse gene signatures

Viruses 2022

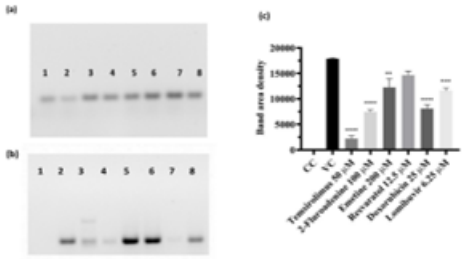
Anti CHIKV activity of fourteen FDA-approved drugs was investigated by *in vitro* and *in silico* approaches. The findings showed that nine compounds, viz., temsirolimus, 2-fluoroadenine, doxorubicin, felbinac, emetine, lomibuvir, enalaprilat, metyrapone and resveratrol exhibit anti CHIKV activity. Furthermore, *in silico* molecular docking studies revealed that these drugs can bind to structural protein targets such as envelope protein, and capsid, and nonstructural proteins NSP2, NSP3 and NSP4 (RdRp). Findings from *in vitro* and *in silico* studies reveal that these drugs can suppress the infection and replication of CHIKV and further *in vivo* studies

Among the drugs which showed anti CHIKV activity in the present study, resveratrol, doxorubicin, lomibuvir and enalaprilat were also reported to exert anti-DENV activity ***(Punekar et al., 2022).*** These drugs might be useful in regions where both viruses are endemic and need to be prioritized. Apart from the drugs with anti-DENV activity, temsirolimus can be taken forward since, the inhibitory effect against CHIKV was greater compared to other drugs. The results of the current investigation could serve as the foundation for *in vivo* studies that examine the possibility of treating chikungunya fever with FDA-approved drugs by drug repurposing ***(Kasbe et al 2023)***.

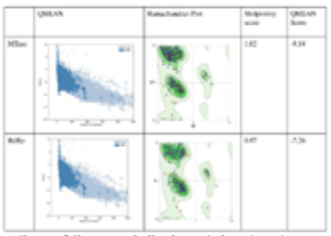
Drug repurposing approach against chikungunya virus: an *in vitro* and *in silico* study



Effect of enalaprilat against virus attachment and entry



Effect of different drugs on CHIKV infection under posttreatment condition using western blot (A) b-actin expression in the cell lysates. (B) Antigens probed with the anti-CHIKV MAb

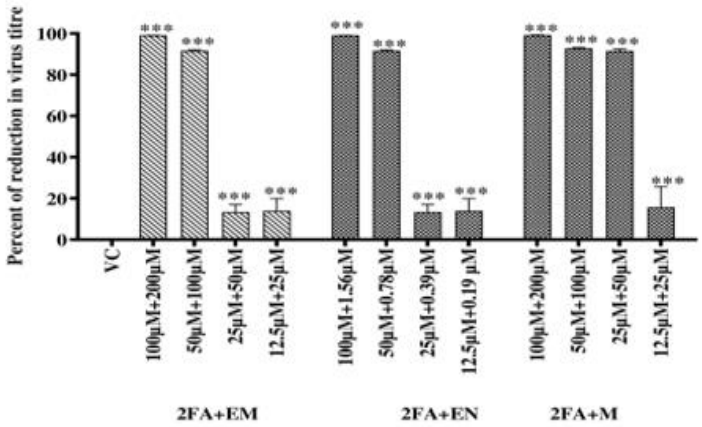


Evaluation of the modelled protein structures of CHIKV Methyl transferase (MTase) and RdRp using ProSA.

Front. Cell. Infect. Microbiol. 2023

To explore the antiviral activity of a combination of repurposed drugs that were reported to have anti-CHIKV activity, the effect of different combinations of six effective drugs (2-fluoroadenine, emetine, lomibuvir, enalaprilat, metyrapone and resveratrol) at their non-toxic concentrations against CHIKV under post infection treatment conditions in Vero cells have been explored. The results revealed that the combination of 2-fluoroadenine with either metyrapone or emetine or enalaprilat exerted inhibitory activity against CHIKV under post-infection treatment conditions. The effect of these drug combinations was additive in nature compared to the effect of the individual drugs. The results suggest an additive anti-viral effect of these drug combinations against CHIKV. The findings could serve as an outline for the development of an innovative therapeutic approach in the future to treat CHIKV-infected patients.

Reduction of virus infectious particles with Metyrapone, Emetine & Enalaprilat against chikungunya virus



The percent reduction:

- ~99% in infected cultures treated with maximum non-toxic dose of different drugs in each combination
- ~90% in infected cultures treated with half the highest non-toxic concentration of different drug in each combination.

Virol. J. 2024

Summary:

Development of novel antivirals against dengue and chikungunya viruses

Dengue (DENV) and chikungunya (CHIKV) viruses and their co-infections have emerged as major public health threats in tropical and sub-tropical regions. In case of both viral infections, initial viral load has been reported to be contributor to disease severity. With the lack of effective vaccines and antivirals, there is a need of effective drug with anti-dengue and anti-chikungunya activity. Using *in vitro* cell culture methods, our studies have identified anti-dengue activity of bioactive compounds such as mangostin, chebulinic acid and carpaine from medicinal plants, plant extracts of *P. alba, A heyneanus, B monnieri, C papaya, V negundo, Sauropus androgynus L. Merr, Ocimum basilicum.* (Patil et al 2021, Thomas et al 2022, Alagarasu et al 2023; Alagarasu et al 2022, Joshi et al 2022, Joshi et al 2023), silver nanoparticles and supercritical fluid extract of *Carica papaya* leaves formulations (Patil et al 2022). Anti-CHIKV activity of bioactive compounds from medicinal plants (mangostin and arctigenin) (Panda et al 2021; Shukla et al., 2024) and plant extracts of *P. alba, A. heyneanus, B. monnieri, C. papaya, C. maxima* (Alagarasu et al 2022), papaya leaves in powder form (Patil et al 2022) and stearylamine (Jeengar et al 2021) have been reported. The study of using zinc exert anti-CHIKV activity at entry and post entry stages of the virus life cycle, while magnesium salt affect CHIKV at post entry stages (Davuluri et al 2024b). Using systems biology approach, we identified nine repurposed drugs viz., temsirolimus, 2-fluoroadenine, doxorubicin, felbinac, emetine, lomibuvir, enalaprilat, metyrapone and resveratrol to exert anti CHIKV activity and resveratrol, doxorubicin, lomibuvir, elvitegravir, and enalaprilat, to exert significant anti-DENV activity (Punekar et al 2022, Kasbe et al 2023). Repurposed drugs in combinations showed that 2-Fluoroadenine with metyrapone or emetine or enalaprilat inhibited chikungunya in an additive manner compared to individual drugs (Davuluri et al 2024a). Novel rhodamine type Anthrone Spiro-lactam analogues showed anti-dengue and anti-chikungunya activity (Darole et al 2023).

Pioneering work done in the area of RNA interference agent (siRNA) for the inhibition of chikungunya virus which was published (Parashar et al, 2013, Parashar & Cherian 2014, Parashar & Cherian 2016) and patents granted in **US (2017), China (2019) Europe (2019), Australia (2021) & India (2021)**. The utility of solid lipid nanoparticles in delivery of antiviral siRNA in *in-vitro* and *in-vivo* model systems was demonstrated. Nanoparticles containing the siRNA approach can be considered for developing a delivery system for the treatment of other viral disease (Jeengar et al 2022). Another delivery system ZIF-C, for siRNA enhances the antiviral activity of chikungunya virus E2 and nsP1 genes directed siRNAs (Tagore et al 2022). The findings from the studies may provide a basis for the future development of a novel therapeutic strategy to treat patients infected with dengue and chikungunya virus. Identified promising compounds and a preclinical study of some compounds provides evidence to support further clinical trials.

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Patent granted*:*

"RNAi agent for inhibition of Chikungunya virus"

-United States Patent (No. US 9574195, Grant date: 21st Feb 2017).
-Chinese Patent (No. ZL201480037556.6, Grant date: 22nd Oct 2019).
-European Patent (No. EP 3017046, Grant date: 11th Sep 2019).
-Australian patent (No. 2014285701, Grant date: 15th July 2021).
-Indian patent (No. 371495, Grant date: 08th July 2021).

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