

In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)

S.No.	Details of publications	
	Small interfering RNA	
1	Parashar D, Paingankar MS, Kumar S, Gokhale MD, Sudeep AB, Shinde SB, Arankalle VA. Administration of E2 and NS1 siRNAs inhibit chikungunya virus replication in vitro and protects mice infected with the virus. PLoS Negl Trop Dis. 2013;7(9):e2405. Published 2013 Sep 5. doi:10.1371/journal.pntd.0002405	<p>We evaluated the efficacy of the siRNAs against ns1 and E2 genes of Chikungunya virus (CHIKV) both <i>in vitro</i> and <i>in vivo</i>. Four siRNAs each, targeting the E2 (Chik-1 to Chik-4) and ns1 (Chik-5 to Chik-8) genes were designed and evaluated for efficiency in inhibiting CHIKV growth in vitro and in vivo. Chik-1 and Chik-5 siRNAs were effective in controlling CHIKV replication in vitro as assessed by real time PCR, IFA and plaque assay. CHIKV replication was completely inhibited in the virus-infected mice when administered 72 hours post infection. The combination of Chik-1 and Chik-5 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.</p> <p><u>Patent granted:</u></p> <p>“RNAi agent for inhibition of Chikungunya virus”</p> <ul style="list-style-type: none"> • United States Patent granted on 21st Feb 2017 and patent no. allotted is US 9574195. • Chinese Patent granted on 22nd Oct 2019 and patent no. is ZL201480037556.6 • European Patent granted on 11th Sep 2019 and patent no. is EP 3017046 • Australian patent granted on 15th July 2021 and patent no. is 2014285701 • Indian patent granted on 08th July 2021 and patent no. assigned is 371495
2	Jeengar MK, Kurakula M, Patil P, More A, Sistla R, Parashar D* . Effect of Cationic Lipid Nanoparticle Loaded siRNA with Stearylamine against Chikungunya Virus. Molecules. 2022;27(4):1170. Published 2022 Feb 9. doi:10.3390/molecules27041170.	Cationic lipids are promising for designing safe non-viral vectors and are beneficial in treating chikungunya. In this study, nanodelivery systems (hybrid polymeric/solid lipid nanoparticles) using cationic lipids (stearylamine, C9 lipid, and dioctadecylamine) and polymers (branched PEI-g-PEG -PEG) were prepared, characterized, and complexed with siRNA. The four developed delivery systems (F1, F2, F3, and F4) were assessed for stability and potential toxicities against CHIKV. In comparison to the other nanodelivery systems, F4 containing stearylamine (Octadecylamine; ODA), with an induced optimum cationic charge of 45.7 mV in the range of 152.1 nm, allowed maximum siRNA complexation, better stability, and higher transfection, with strong inhibition against the E2 and NS1 genes of CHIKV. The study concludes that cationic lipid-like ODA with ease of synthesis and characterization showed maximum complexation by structural condensation of siRNA owing to high transfection alone. Synergistic inhibition of CHIKV along with siRNA was demonstrated in both <i>in vitro</i> and <i>in vivo</i> models. Therefore, ODA-based cationic lipid nanoparticles can be explored as safe, potent, and efficient nonviral vectors overcoming siRNA in vivo complexities against chikungunya.
3	Tagore R, Alagarasu K, Patil P,	Small interfering RNA (siRNA) mediated gene silencing of CHIKV

	Pyreddy S, Polash SA, Kakade M, Shukla R, Parashar D* . Targeted <i>in vitro</i> gene silencing of E2 and nsP1 genes of chikungunya virus by biocompatible zeolitic imidazolate framework. Front Bioeng Biotechnol. 2022;10:1003448. Published 2022 Dec 14. doi:10.3389/fbioe.2022.1003448	structural and non-structural genes serves as a potential antiviral strategy. The therapeutic efficiency of siRNA can be improved by using an efficient delivery system. Metal-organic framework biocomposites 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.
Antivirals (Extracts/Compounds)		
4	Alagarasu K, Patil P, Kaushik M, Chowdhury D, Joshi RK, Hegde HV, Kakade MB, Hoti SL, Cherian S, Parashar D* . <i>In Vitro</i> Antiviral Activity of Potential Medicinal Plant Extracts Against Dengue and Chikungunya Viruses. Front Cell Infect Microbiol. 2022;12:866452. Published 2022 Apr 7. doi:10.3389/fcimb.2022.866452.	Dengue and chikungunya are two important mosquito-borne infections which are known to occur extensively in tropical and subtropical areas. Presently, there is no treatment for these viral diseases. In vitro antiviral screening of 25 extracts prepared from the plants of <i>Vitex negundo</i> , <i>Plumeria alba</i> , <i>Ancistrocladus heyneanus</i> , <i>Bacopa monnieri</i> , <i>Anacardium occidentale</i> , <i>Cucurbita maxima</i> , <i>Simarouba glauca</i> , and <i>Embelia ribes</i> 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. Maximum nontoxic concentrations of the chloroform, methanol, ethyl acetate, petroleum ether, dichloromethane, and hydroalcoholic extracts of eight plants were used. The antiviral activity was assessed by focus-forming unit assay, quantitative real-time RT-PCR, and immunofluorescence assays. Extracts from <i>Plumeria alba</i> , <i>Ancistrocladus heyneanus</i> , <i>Bacopa monnieri</i> , and <i>Cucurbita maxima</i> showed both anti-DENV and CHIKV activity while extract from <i>Vitex negundo</i> 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. The present study had identified the plant extracts with anti-dengue and anti-chikungunya activities, and these extracts can be further characterized for finding effective phytopharmaceutical drugs against dengue and chikungunya.
5	Joshi RK, Agarwal S, Patil P, Alagarasu K, Panda K, Prashar C, Kakade M, Davuluri KS, Cherian S, Parashar D* , Pandey KC, Roy S. Effect of <i>Sauropus androgynus</i> L. Merr. on dengue virus-2: An in vitro and in silico study. J Ethnopharmacol. 2023;304:116044. doi:10.1016/j.jep.2022.116044.	<i>Sauropus androgynus</i> L. Merr. (Euphorbiaceae) commonly known as “multigreen” and “multivitamin” is consumed as a vegetable and used in traditional medicine to relieve fever. This <i>in vitro</i> study is aimed to explore the activities of the lipophilic fraction of the leaves of <i>S. androgynus</i> (LFSA) against DENV, CHIKV and malaria parasite. The LFSA was analyzed by using GC-FID and GC-MS. The antiviral activity of LFSA was studied using the Vero CCL-81 cell line. The cytotoxicity assay was performed using 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide. Focus forming unit, cell-based immunofluorescence assays, and quantitative RT-PCR, were used to determine and confirm antiviral activity against DENV and CHIKV. The antiparasitic activity of LFSA was carried out against <i>P. falciparum</i> strain 3D7 grown in fresh O+

		human erythrocytes culture. 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-2. In-silico results suggested that β-sitosterol may block the viral entry by inhibiting the fusion process.
6	Alagarasu K, Puneekar M, Patil P, Kasabe B, Kakade M, Davuluri KS, Cherian S, Parashar D* . Effect of carpaine, a major alkaloid from <i>Carica papaya</i> leaves, on dengue virus-2 infection and replication-an in-vitro and in-silico study [published online ahead of print, 2023 Jan 1]. <i>Phyther Res.</i> 2023;10.1002/ptr.7715. doi:10.1002/ptr.7715.	The findings of the present study 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 <i>in-vitro</i> and <i>in-silico</i> evidence of anti-DENV activity of carpaine against DENV-2 in Vero CCL-81 cells.
7	Panda K, Alagarasu K, Patil P, Agrawal M, More A, Kumar NV, Mainkar PS, Parashar D* , Cherian S. In Vitro Antiviral Activity of α-Mangostin against Dengue Virus Serotype-2 (DENV-2). <i>Molecules.</i> 2021;26(10):3016. Published 2021 May 19. doi:10.3390/molecules26103016.	Approved antiviral therapies or vaccines for the treatment or prevention of DENV infections are not available. In the present study, natural compounds were 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 antiviral activity was determined by foci forming unit assay, quantitative RT-PCR and cell-based immunofluorescence assay. A complete inhibition of DENV-2 was observed at 8 µM under the co-treatment condition. The possible inhibitory mechanism of α-Mangostin was also determined by docking studies. The molecular docking experiments indicate that α-Mangostin can interact with multiple DENV protein targets such as the NS5 methyltransferase, NS2B-NS3 protease and the glycoprotein E. 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.
8	Patil P, Agrawal M, Almelkar S, Jeengar MK, More A, Alagarasu K, Kumar NV, Mainkar PS, Parashar D* , Cherian S. <i>In vitro</i> and <i>in vivo</i> studies reveal α-Mangostin, a xanthanoid from <i>Garcinia mangostana</i> , as a promising natural antiviral compound against chikungunya virus. <i>Virol J.</i> 2021;18(1):47. Published 2021 Feb 28. doi:10.1186/s12985-021-01517-z.	As diverse natural phenolic compounds have been shown to possess antiviral activities, we explored the antiviral activity of α-Mangostin, a xanthanoid, against CHIKV infection. The <i>in vitro</i> prophylactic and therapeutic effects of α-Mangostin on CHIKV replication in Vero E6 cells were investigated by administering it under pre, post and cotreatment conditions. The antiviral activity was determined by foci forming unit assay, quantitative RT-PCR and cell-based immunofluorescence assay. The molecular mechanism of inhibitory action was further proposed using in silico molecular docking studies. In vitro studies revealed that 8 µM α-Mangostin completely inhibited CHIKV infectivity under the cotreatment condition. CHIKV replication was also inhibited in virus-infected mice. This is the first <i>in vivo</i> study

		<p>which clearly showed that α-Mangostin is effective <i>in vivo</i> 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.</p>
9	<p>Jeengar MK, Kurakula M, Patil P, More A, Sistla R, Parashar D*. Antiviral activity of stearylamine against chikungunya virus. <i>Chem Phys Lipids</i>. 2021;235:105049. doi:10.1016/j.chemphyslip.2021.105049.</p>	<p>The present study was aimed to investigate the antiviral activity of Stearylamine (SA) against CHIKV in both <i>in vitro</i> and <i>in vivo</i>. The antiviral activity of SA was determined by foci forming unit assay, quantitative RT-PCR and cell-based immune-fluorescence assay. Further <i>in vivo</i> studies were carried out to see the effect of SA treatment in CHIKV infected C57BL/6 mice. The anti-CHIKV activity was evaluated using qRT-PCR in serum and muscle tissues at different time points and by histopathology. In vitro treatment with SA at a concentration of 50μM showed a reduction of 1.23 ± 0.19 log₁₀ FFU/mL at 16 h and 1.56 ± 0.12 log₁₀ FFU/mL at 24 h posttreatment by FFU assay. qRT-PCR studies indicated that SA treatment at 50μM concentration showed a significant reduction of 1.6 ± 0.1 log₁₀ and 1.27 ± 0.12 log₁₀ RNA copies when compared with that of virus control at 16 and 24 h post incubation. Treatments in the C57BL/6 mice model revealed that SA at 20 mg/kg dose per day up to 3, 5 and 7 days, produced stronger inhibition against CHIKV indicating substantially decrease viral loads and inflammatory cell migration in comparison to a dose of 10 mg/kg. This first <i>in vivo</i> study clearly indicates that SA is effective by significantly reducing virus replication in serum and muscles. As a next-generation antiviral therapeutic, these promising results can be translated for the use of SA to rationalize and develop an ideal delivery system alone or in combination against CHIKV.</p>
Repurposed drugs		
10	<p>Kasabe B, Ahire G, Patil P, Puneekar M, Davuluri KS, Kakade M, Alagarasu K, Parashar D*, Cherian S. Drug repurposing approach against chikungunya virus: an <i>in vitro</i> and <i>in silico</i> study. <i>Front Cell Infect Microbiol</i>. 2023;13:1132538. Published 2023 Apr 27. doi:10.3389/fcimb.2023.1132538</p>	<p>In the present study, anti CHIKV activity of fourteen FDA-approved drugs was investigated by <i>in vitro</i> and <i>in silico</i> approaches. Focus-forming unit assay, immunofluorescence test, and quantitative RT-PCR assay were used to assess the <i>in vitro</i> inhibitory effect of these drugs against CHIKV in Vero CCL-81 cells. The findings showed that nine compounds, viz., temsirolimus, 2-fluoroadenine, doxorubicin, felbinac, emetine, lomibuvir, enalaprilat, metyrapone and resveratrol exhibit anti chikungunya activity. Furthermore, <i>in silico</i> molecular docking studies performed by targeting CHIKV structural and non-structural proteins 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 <i>in vitro</i> and <i>in silico</i> studies reveal that these drugs can suppress the infection and replication of CHIKV and further <i>in vivo</i> studies followed by clinical trials are warranted.</p>