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Evaluation of medicinal herbs for Anti-CHIKV activity.

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ABSTRACT

Chikungunya, a mosquito-borne viral disease is now a global public health problem. In tropical countries such as India, periodic chikungunya outbreaks can occur due the high prevalence of the mosquito vector, circulation of virus and the high population density. To curtail the virus in outbreak situation, a ready to use drug for chikungunya is necessary. Using the literature mentioned plant extracts, we used four assays to screen and identify indigenous plants with CHIKV inhibitory activity. Our results showed that the aqueous extract of five plant extracts exhibited anti-CHIKV activity by inhibiting viral attachment, four plant extracts exhibited replication inhibition through inhibition of helicase activity, two plants showed inhibition of protease activity. Two plant extracts showed both viral attachment inhibition and replication inhibition and also exhibited dose dependent response in virus replication inhibition assay. These findings warrant further investigation to standardize these plant extracts as antiviral formulation for chikungunya infection.

1. Introduction

In 1952, Chikungunya virus (CHIKV) was found to be the cause of the disease in humans. The disease symptoms include fever with prolonged joint pain. Globally, Scientists from various labs are trying to combat this disease by various means which include mosquito repellents, vaccines, antibodies, drugs from various sources (Ray et al., 2012). All the antiviral prospects are at different stages of development. Various antiviral strategies include inhibitors of viral entry like chloroquine, arbidol and its derivatives, phenothiazines, epigallocatechin gallate, inhibitors of viral protein translation like small interfering RNAs, harringtonine and homoharingtonine, inhibitors of viral genome replication like ribavirin, 6-azauridine, mycophenolic acid, favipravir. All these drugs are yet to reach the market and they have their own pros and cons associated with them (Abdelnabi et al., 2016).

In recent times, due to increased global travel, spread of CHIKV in Americas was also reported. Recent study quantified and provided the first epidemiological evidence that chikungunya epidemic characteristics are strongly influenced by CHIKV lineage (Bustos Carrillo et al., 2018). Symptomatic chikungunya illness can range from mild fever to severe and prolonged joint pains and arthralgia (Raghavendhar et al., 2016). Severe chronic arthralgia and/or arthritis that can last months to years following the initial infection is the prominent feature of CHIKV infection (Goupil and Mores, 2016). In case of chikungunya, the appearance of virus in the serum coincides with fever onset, with titers reaching 10^9 RNA copies/ml, at the peak of the febrile phase and the titers drop down with the simultaneous increase in titres of antibodies that result in certain features like skin rash, myalgia and joint pains (Chusri et al., 2014; Simon et al., 2011). Currently only symptomatic treatment is available and it is necessary to have specific antivirals.

CHIKV is a positive-sense single-stranded RNA virus and classified as an Arbovirus or Arthropod Borne Virus because of its transmission via mosquitoes. Phylogenetic analyses of genome sequences identified three major genetically distinct CHIKV lineages, West African (WA); East, Central, and South African (ECSA); and Asian. Another distinct sub lineage arising from the ECSA lineage during the Indian Ocean outbreak is called the Indian Ocean lineage (IOL) (Langsjoen et al., 2018). Currently IOL lineage of CHIKV is prevalent in India from 2010 (Raghavendhar et al., 2016). Controlling the spread of chikungunya continues to be an uncontrollable problem due to the inability to eradicate the vector mosquitoes and the lack of safe, potent preventive vaccine (Weaver et al., 2012). Despite numerous hurdles, persistent heterogeneous strategic efforts over the years (since the late 1960s) have resulted in the development of several live attenuated chikungunya vaccine candidates, one of which has recently qualified to enter phase II clinical trials and is expected to be licensed in the near future (Schwameis et al., 2015). The challenges being faced in chikungunya vaccine development have emphasized the need for antiviral drugs and instigated new efforts in this direction.

Due to increased public health concern around the world, there is an

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urgent need to develop an antiviral drug by looking at various possibilities (Raghavendhar et al., 2016). The use of natural products as medicines for treating various human ailments been described throughout history (Dias et al., 2012). India is well known for ayurvedic practices using herbs with medicinal value. Ayurveda prescribes syrups extracted from medicinal herbs for a various illnesses (Katiyar et al., 2012). The current work was undertaken with the objective of exploring the possibility of identifying anti-CHIKV activity that may be associated with indigenous medicinal herbs. To choose plants likely to provide useful leads, the study utilized names of plants mentioned for chikungunya like symptoms in the ayurvedic literature (Lavekar and Padhi, 2009). Though chikungunya as such is not described in Ayurveda literature, there are illnesses identified by symptoms that can be correlated with some of the clinical manifestations of chikungunya fever (CHIKF).

Usage of plant extracts for disease treatment is a usual practice followed by Indian Ayurveda practitioners (Lavekar and Padhi, 2009). However, ayurvedic drugs sold for chikungunya like symptoms are lacking documented proof of action (Powers, 2018). There is strong need to verify these formulations through standard, specific, in-vitro antiviral experiments. This study evaluates various literature reviewed medicinal plants for their anti-CHIKV activity through in-vitro screening experiments that were set-up to identify anti-CHIKV activity and presents data about plant extracts that showed inhibitory potency against CHIKV.

2. Materials and methods

2.1. Cells and virus

The monkey kidney cells, Vero cells were maintained in Dulbecco's Modified Eagle medium (DMEM), supplemented with 10% FBS, in a 5% CO_2 humidified incubator, at 37 °C. African CHIKV strain S-27 was used in this study. This was propagated in Vero cells and titrated using a standard plaque assay as described below.

2.2. Preparation of plant extracts

Plants shortlisted for screening are indicated in Table 1. These were procured through local suppliers and authenticated by the resident ayurvedic doctor at IIT hospital, New Delhi, India. Water extracts were prepared from dried powder of the plant parts used. Two grams of powder was mixed in 10 ml of distilled water and kept at 50 °C overnight and centrifuged at 1000 rotations/10 min. Supernatant was collected, filtered, and concentrated at low pressure and temperature, and dried in a vacuum oven at room temperature (RT) for 16–18 h. The resultant material was dissolved in MilliQ water at 1 mg/ml and stored at −20 °C and used for further experiments.

2.3. Plaque assay

The titre of infectious CHIKV in virus stocks were determined using a standard plaque assay as described previously (Kaur et al., 2016). Briefly, Vero cell monolayers in 6 well plates were infected in duplicate with serial 10-fold dilutions (prepared in DMEM + 2%FBS) of the virus-containing samples (200µl/well). Mock-infections were performed in parallel using an equivalent volume of virus diluent alone. Virus adsorption was carried out for 2 h at 37 °C. After aspirating the inoculum cells were overlaid with DMEM + 6%FBS containing 0.3% Agarose (2 ml/well), and incubated for 4 days (37 °C, 5% CO_2). On day 4 post-infection, the overlay was removed and the cells fixed with 3% formaldehyde solution (1 ml/well). Fixed cells were washed and stained with 0.05% (w/v) crystal violet solution in 20% ethanol. Revealed plaques were counted to determine the virus titre, expressed as plaque-forming units (PFUs)/ml.

2.4. Plaque reduction assay

The initial antiviral screening assay, was based on the plaque reduction neutralization test (PRNT) described earlier (Hsuan et al., 2009). Briefly, confluent monolayers of Vero cells were seeded in 24-well plates (5×10^5 cells/well), a day in advance. 100 µl of extracts at 10 µg/mL and 100 µl of 100 pfu/ml CHIKV were pre-incubated overnight at 4°c and added to the monolayer Vero cells and incubated under 5% CO_2 at 37 °C. After 2 h The incubating solution contain virus and extract was discarded and 1 mL 0.3% agarose in DMEM containing 2% FBS added to the cells and the plates incubated under 5% CO_2 for 48 h at 37 °C and processed thereafter as described for the plaque assay above. Cells treated virus but not with plant extract served as the control for plaque formation and those treated with extract alone served as control for cytotoxicity of used concentration while those not treated with either virus or extract served as control for healthy growing cells. The % plaque inhibition concentration was determined by the following formula (Liu et al., 2012), % inhibition = $[1 - (pfu_{exp}/pfu_{control})] \times 100$, where pfu_{exp} = plaque formation by extract-and virus-treated cells and $pfu_{control}$ = plaque formation by virus-treated cells.

2.5. Virus replication inhibition assay

Vero cells in 24-well plates were infected with CHIKV (100 µL of 100 pfu). After 2 h of adsorption under 5% CO_2 at 37 °C, the virus inoculum was aspirated, and then fed with complete medium containing the herbal extracts of either 25, 50, 100, or 200 µg/mL final

Table 1
Screening of plant extracts for anti-CHIKV activity.

S·NO	Common name of Plant	Scientific Name	Part used	Plaque reduction assay	Helicase assay	Protease assay
1	Katuki	Picrorhiza *kurroa*	Rhizome	+	+	–
2.	Shyama Tulasi	Ocimum *tenuiflorum*	Whole plant	+	+	+
3.	Haritaki	Terminalia *chebula*	fruit	+	+	+
4.	Giloi Guduchi	Tinospora *cordifolia*	stem	–	–	–
5.	Salaki	Boswellia *serrata*	Resin	–	–	–
6.	Sunti	Zingiber *officinale*	Rhizome	–	+	–
7.	Amalaki	Phyllanthus *emblica*	Fruit	–	–	–
8.	Guggulu	Commiphora *wightii*	RESIN	+	–	–
9.	Haridra	Curcuma *longa*	Rhizome	–	–	–
10.	Manjista	Rubia *cordifolia*	Stem	–	–	–
11.	Nimba	Azadirachta *indica*	Bark	–	–	–
12.	Rasna	Pluchea *lanceolate*	Whole plant	–	–	–
13.	Nirgundi	Vitex *negundo*	Fruits, leaves	–	–	–
14.	Salparni	Desmodium *gangeticum*	Whole plant	–	–	–
15.	Devdaru	Cedrus *deodara*	bark	+	–	–

concentration in triplicates. After 24 h of exposure to the extract, the incubating solution was discarded and overlaid with growth medium containing 0.3% agarose and plaques developed 4 days later were counted and the %inhibition was calculated as described in plaque reduction assay.

2.6. Protein expression and purification

The expression construct of nsP2FL(798 aa) or truncated nsP2 (429aa) was made into the modified pET28 vector between NdeI and HindIII sites. The resulting expression construct encoded for nsP2FL (798 aa) or truncated nsP2(429aa) and an N-terminal His-tag, were separated by a cleavage site for precision protease. N-terminally His-tagged nsP2 was expressed in *Escherichia coli* BL21 (DE3) Rosetta RIL cells with the auto induction method (Studier, 2005). The cells were first cultured for 2 h at +37 °C, then cooled on ice, and further cultured at +18 °C for 18 h. The cells were harvested by centrifugation, and the pellet was resuspended in buffer A (50 mM Tris pH 7.5, 500 mM NaCl, 10% glycerol, 10 mM Imidazole) containing protease inhibitors 1 mM DTT, 1 mM PMSF and DNAseI 0.001 mg/ml with 5 mM MgCl2. The cells were lysed by adding lysozyme having final concentration 1 mg/ml and disrupted by sonication on ice, and the lysate was clarified by centrifugation at 45000*g* for 20 min. The clear lysate was applied onto a Ni-NTA column pre-equilibrated with buffer A. The column was washed with 200 ml of buffer A, and nsP2 protein was eluted with 250 mM imidazole in buffer A. The fractions were analysed on SDS-PAGE for purity. Fractions containing nsP2 proteins were pooled and diluted 5 times with buffer B (50 mM Tris pH 7.5 and 10% glycerol) and applied onto a cation exchanger SPFF column pre-equilibrated with buffer C (50 mM Tris pH 7.5, 100 mM NaCl, 10% glycerol). The column was washed with 200 ml of buffer C, and nsP2 protein was eluted with a linear gradient of 50% buffer D (50 mM Tris pH 7.5, 1 M NaCl, 10% glycerol).

The fractions were again analysed on SDS-PAGE for purity. Fractions containing nsP2 proteins were pooled and cleaved with recombinant Precision protease at +4 °C overnight.

The overnight cleaved sample was reapplied on Ni-NTA column pre-equilibrated with buffer A. The flow through has been collected, concentrated, filtered, and injected into a pre-equilibrated size exclusion column (Superdex S200 16/60 column). Size exclusion chromatography was carried out in buffer E (50 mM Tris pH 7.5, 300 mM NaCl, 10% glycerol and 1 mM DTT), at a flow rate of 1 ml/min. The peak fractions having nsP2 FL (or truncated nsP2(429aa)) were analysed on SDS-PAGE, and the fractions of the apparent protein peak were pooled and concentrated, flash frozen, kept in −80 °C for all biochemical studies.

2.7. Helicase assay

The RNA unwinding assay was performed in 10 µl reaction mixtures containing indicated amounts of 50 nM of nsP2 proteins and buffer (20 mM Tris-HCl pH 7.5, 50 mM KCl, 5.0 mM $MgCl_2$, 1.0 mM DTT, 5% sucrose, 0.1 mg/ml BSA), 0.10 U/ml RNasin (Promega)), 2 mM ATP and 50 nM of double-stranded RNA substrate. All reagents were kept on ice and preincubated at room temperature for 10 min before initiation of the reactions. The substrate had shorter strand of 30 nt, labelled at 5′ with 56-FAM, and the longer RNA strand had a 40 nt 3′ overhang, 30 nt complementary to the shorter strand and a 10 nt 3′ overhang. The substrate was prepared with oligonucleotide 1 which is fluorescently labelled with 5′ FAM: CACCGGCAAGGUCGCUGUUCAAUACAUGCA and 3' unlabelled oligonucleotide 2: CCAGUCGGAUUGCAUGUAUUGA ACAGCGACCUUGCCGGUG. The mixtures were prepared in a final volume of 10 µl of buffer containing 10 mm HEPES, pH 7.2 and 20 mm KCl; heated to 95 °C; and allowed to slowly cool to 22 °C. The prepared dsRNA substrate was purified using 12% Native electrophoresis system. For the effect of medicinal plants on helicase activity, the crude water extracted composite mixtures 10 µg with volume 1 µl of 10 µg/ml was added in the reaction mixture. After incubation for 30 min with or without different plant composite mixture at 25 °C the reaction was stopped by addition of 2.0 µl of 2% SDS and 100 mM EDTA. Helicase reaction species were separated by native acrylamide electrophoresis (12% acrylamide with 60:1 acrylamide to bis-acrylamide and run in 0.25 X TBE at 150 V for 2 h), detected by fluorescence using a Typhoon 9410 variable mode imager (GE Healthcare) and analysed by ImageJ (http://imagej.nih. gov/ij/) (Mukherjee et al., 2012). The helicase reactions are representative of three or more experiments carried out under similar conditions.

2.8. FRET based protease inhibition assay

The protease activity was established for the purified protein by fluorescence resonance energy transfer based assay(Saha et al., 2018), with some modifications. The FRET-based peptide substrate was employed with sequence (Nma-RAGCAPSYRK-Dnp) containing sessile site for nsP2 protease between nsp2-3 junction was synthesized commercially (Bio Cell Corporation, New Delhi, India). In the assay, full length nsP2FL (798aa) was used for activity measurement however truncated CHIKV nsP2 (429aa) was used as a negative control. The synthetic protease substrate has Nma 2-(*N*-methylamino) benzoyl fluorophore group attached at the amino terminus and 2, 4-dinitrophenyl group attached to the carboxyl terminus of Lysine (K) residue as quencher. The peptide was dissolved at mM concentration in dimethyl formamide (Sigma Aldrich) and stored at −80 °C deep freezer. For protease assay standardization, the substrate at different concentration ranging from 0.5 to 50 µM in assay buffer (20 mM Bis-Tris-Propane, pH 8.0) was added to Nunc 96 Well black plates with 1 µM nsP2 protease diluted in the same buffer in total volume of 100 µl in triplicates. For measurements we used 10 µM of substrate and 1 µM of enzyme nsP2FL or nsP2(429), fluorescence readings were measured at 5 min interval for 1 h using Cary Eclipse fluorescence spectrophotometer (Agilent technologies) Multimode plate reader at an excitation wavelength (λex) of 340 nm and an emission wavelength (λem) of 440 nm. Varying concentrations of the medicinal plants were tested for their protease inhibitory potential by incubating with nsP2 protein for 60 min at room temperature before adding the peptide substrate to the mixture. Later, the enzymatic reaction was incubated with 1 µg of different plants composite extracts separately for 60 min at room temperature. The increase in intensity of fluorescence emission related to protease activity and relative decrement of the same in presence of inhibitor quantified as protease inhibitory potential against CHIKV full length nsP2.

2.9. Bio-safety clearance

All invitro experiments were performed as per the Institute Biosafety committee guidelines.

2.10. Statistical analysis

Stata 12.0 was used for statistical analysis. In case of duplicates or triplicates, average of values was taken as final value. Significant difference ($p < 0.05$) determined by ANOVA.

3. Results

3.1. Plaque reduction assay

The first assay was designed to identify herbal extracts that had the ability to block CHIKV from entering susceptible cells. The possible outcomes of this assay were, the extract could be cytotoxic (compromised monolayer), inactive (no reduction in plaques in comparison to control infection), or active (reduced number of plaques). Multicomponent plant extract (standard concentration 10 µg/mL)

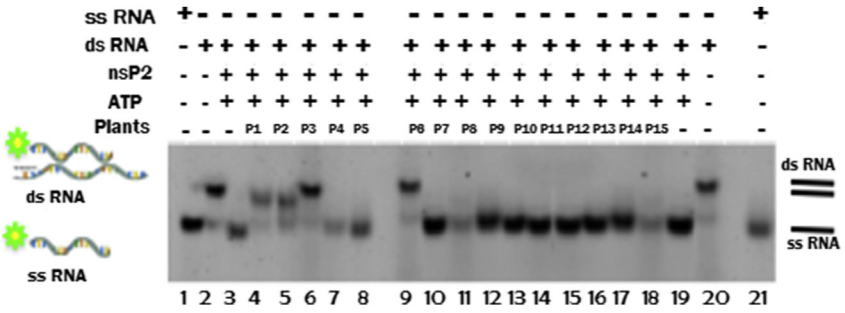

Fig. 1. Helicase activity assay. Lane 1 is single-stranded 30 bp oligonucleotide and Lane 2 is a fully duplex RNA of the 40 bp length with 10 base overhang. CHIKV nsP2 displayed unwinding activity in presence of ATP (Lane 3), which has been inhibited in presence of 10 µg of different plants P1, P2, P3 and P6 (Lane 4,5,6 and 9). The effect of plants alone has no effect on helicase activity.

which showed plaque inhibition without causing cytotoxicity in the drug alone wells was considered to be active as a CHIKV inhibitor. 15 herbal extracts were screened for their antiviral effect by plaque reduction assay. Five plants extracts (10 µg/ml) inhibited CHIKV plaque formation at more than 80% level (Table-1).

3.2. Helicase assay

All the 15extracts were then screened for enzyme activity inhibition assay. Using denaturing PAGE band shift assay, we established that CHIKV nsP2 helicase displays unwinding activity on an overhang 10 bp duplex RNA (Fig-1). This assay identified few plants P1, P2, P3 and P6 as potential inhibitor for chikungunya virus replication targeting RNA helicase activity which shows failure in unwinding dsRNA to ssRNA (Table-1).

3.3. Protease assay

For assessing the inhibition effect on chikungunya virus, we have used nsP2 full length protease activity using FRET based assay system. We used fluorescence intensity (FI) based synthetic peptide substrate with sequence (Nma-RAGCAPSYRK-Dnp) containing sessile site for nsP2 protease between nsp2-3 junction for the proteolytic activity assay of CHIKV nsP2 full length. The active CHIKV nsP2protease exhibits the efficient cleavage of the substrate peptide, which separates fluorophore Nma 2-(*N*-methylamino) benzoyl and quencher, 2,4-dinitrophenyl resulting in the increased fluorescence signal. Fluorescence profile for the hydrolysis of the FRET-based substrate with time has been done for optimized reaction. A control reaction was also performed using the same reaction without enzyme, which did not show change in the fluorescence intensity. Finally, experiments were carried out to estimate effect of medicinal plants on CHIKV nsP2 protease activity by carrying out assay with incubating with different medicinal plants. Therefore, a proper reaction was obtained using FRET-based peptide substrate for assessing the proteolytic activity inhibition by change in fluorescence. Two medicinal plants P2 Ocimum *tenuiflorum* and P3 Terminalia *chebula* when 1 µg dry weight in water was incubated with 1 µM of nsP2 FL protein were able to inhibit the increase in fluorescence intensity significantly indicating inhibition of CHIKV nsP2 protease activity (Fig. 2). Further, purified truncated CHIKV nsP2 (429aa) was used as a negative control. CHIKV nsP2 (429aa) was not able to cleave the substrate efficiently indicating that the c-terminal region is crucial for nsP2pro activity. The fluorescence intensity of nsP2 FL is taken as 100% activity and relative decrement of fluorescence intensity has been calculated as inhibition [Fig. 2].

3.4. Virus replication inhibition assay

The herbal extracts that showed positive effect in the initial plaque reduction assay (n = 5), were used in this assay to assess their ability to internalize and inhibit CHIKV within the cell The Vero cells were infected with fixed concentration of CHIKV for 2 h and then treated with either 25, 50, 100, or 200 µg/ml of selected plant extracts to determine

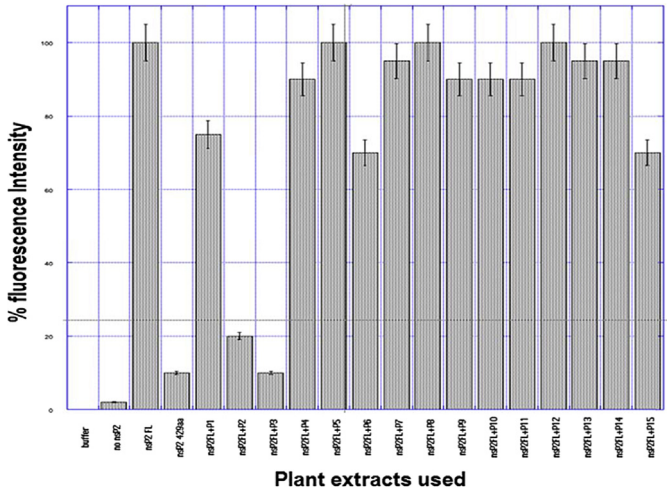

Fig. 2. Protease activity assay. FRET based protease Assay. The bar shows the fluorescence intensity with respect to Substrate and protease reaction. Bar 1, only buffer; Bar 2, without nsP2 protein; Bar3, with nsP2FL; Bar 4, with nsP2429aa; Bar 5–20, with nsp2 and different plants 1–15 respectively.

their effect on virus replication. The results showed that the number of plaques decreased with increase in extract concentration in case of two plant extracts (Ocimum *tenuiflorum*, Terminalia *chebula*), complete inhibition was observed at 200 µg/ml while one extract (Picrorhiza *kurroa*) showed 67% inhibition at higher concentration (200 µg/ml) only and rest two extracts (Commiphora *wightii* and Cedrus *deodara)* showed around 30% inhibition at the higher concentration (200 µg/ml) (Table-2). Significant difference ($p < 0.05$) between the % inhibition effects by every concentration of particular extract was observed by ANOVA.

4. Discussion

Chikungunya continues to be a significant global public health problem, due to the lack of a licensed vaccine or antiviral drug (Ray et al., 2012). Increased global travel contributed to the high rates of disease transmission. Early detection and timely management with specific antivirals can prevent the spread of virus transmission (Raghavendhar et al., 2016). Usually, the level of viremia is generally higher during first four days of illness and gradually drops while progressing towards sero-conversion phase where antibody titres are higher (Ray et al., 2012). Severe manifestations like persistent arthralgia and myalgia may increase the duration of illness (Siva et al., 2019). Antivirals administered during any of these phases should have the ability to inhibit virus attachment, entry, replication and egress processes. Multicomponent plant extracts can be a good source of such inhibitors and were evaluated in this study.

Based on literature search we chose indigenous medicinal plants prescribed for treating chikungunya like symptoms, for anti-CHIKV activity screening (Lavekar and Padhi, 2009). Currently East/Central/

Table 2
Plant extracts used in virus replication inhibition assay.

Plant extract		Concentration used and % inhibition			
Common Name	Scientific name	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Katuki	Picrorhiza *kurroa*	Nil	Nil	38.3%	67%
Shyama Tulasi	Ocimum *tenuiflorum*	28.3%	53.3%	85%	100%
Haritaki	Terminalia *chebula*	23%	51.6%	83.3%	100%
Guggulu	Commiphora *wightii*	Nil	Nil	Nil	31.6%
Devdaru	Cedrus *deodara*	Nil	Nil	Nil	36.6%

Significant difference ($p < 0.05$) between the % inhibition effects by every concentration of particular extract was observed by ANOVA.

South African (ECSA) lineage of CHIKV is prevalent in India (Raghavendhar et al., 2016), hence it is desirable to have an effective antiviral against this circulating lineage. For this purpose plaque reduction assay, virus replication inhibition assay and enzyme activity assays were used to evaluate the antiviral activity of medicinal herbs. Generally, antiviral drug that can stop viral attachment to cells and can also inhibit proteins involved in replication are considered to be effective ones. Plant extracts were screened for viral attachment inhibition by plaque reduction assay using pre-incubated mixture (virus + plant extract) and assessed for replication inhibition using helicase assay and virus replication inhibition assay.

This resulted in the identification of plant extracts that inhibit viral attachment, replication and both. Picrorhiza *kurroa*, Ocimum *tenuiflorum*, Terminalia *chebula*, Commiphora *wightii* and Cedrus *deodara* showed their ability to inhibit CHIKV in the plaque reduction assay format suggests their ability as viral attachment inhibitors. Plant extracts of Picrorhiza *kurroa*, Ocimum *tenuiflorum*, Terminalia *chebula* and Zingiber *officinale* showed inhibition of helicase activity suggesting their potential utility as replication inhibitors. Extracts of Ocimum *tenuiflorum* and Terminalia *chebula* emerged as protease inhibitors. Three plant extracts (Picrorhiza *kurroa*, Ocimum *tenuiflorum*, Terminalia *chebula*) exhibited inhibition activity in two assays (plaque reduction, helicase assay) and two plant extracts (Ocimum *tenuiflorum*, Terminalia *chebula*) exhibited inhibition in all three assays (plaque reduction, helicase, and protease) and exhibited dose dependent response in virus replication inhibition assay.

These preliminary results demonstrated complete antiviral ability of two plant extracts (Ocimum *tenuiflorum*, Terminalia *chebula*) which can be prospectively used in further investigations like animal studies. Structured study using mice can bring herbal remedies for tackling CHIKV infection. Spread of infection during outbreaks can be controlled with potent antiviral herbal interventions.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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