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Anti-Chikungunya and Anti-Inflammatory Therapeutic Potential of *Cynodon dactylon* Aqueous Extract

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Chikungunya is an infectious, mosquito-borne viral disease that poses a significant threat to global public health. No licensed antiviral drugs or vaccines are available to treat CHIKV infection. The present study aims to investigate the in vitro antiviral and anti-inflammatory potential of Cynodon dactylon aqueous extract. FTIR and GC-MS phytochemical studies were used to identify the primary bioactive component. The maximum non-toxic dose of the plant was determined through an MTT assay on Vero cells. Subsequently, the antiviral potential was evaluated through pre-, co-, and post-treatment assays to determine the stage at which the extract is most effective. Cell morphological features were microscopically examined for cytopathic effects, followed by the evaluation of virus titre via the focus-forming unit using culture supernatant. The phytochemical study revealed 24 bioactive chemicals, the most common of which was hexadecanoic acid eicosyl ester, accounting for 24.64% of the total. The maximum non-toxic dose was 62.5 μg/mL, with an IC50 of 420.67 μg/mL. Post-treatment showed the highest antiviral activity, reducing the viral titre from 8.10 (virus control) to 7.36 mean log10 FFU/mL, corresponding to 82.25% inhibition. The anti-inflammatory assay of the plant extract showed a percentage inhibition of 72.38% at 500 μg/ml, compared to the standard diclofenac sodium (75.14%). Medicinal plants and their bioactive compounds serve as a vital source of antimicrobial agents, offering promising alternatives for the development of natural therapeutics. This study demonstrates that Cynodon dactylon can effectively suppress CHIKV-induced infection and inflammation. Furthermore, hexadecanoic acid eicosyl ester has the potential to be developed as an anti-Chikungunya therapeutic candidate; however, it requires further validation in in vivo models.

Keywords: Antiviral; Anti-inflammatory; Chikungunya Virus; Cynodon dactylon; Cytotoxicity; FFU assay.

Chikungunya is a mosquito-borne *Alphavirus* belonging to the *Togaviridae* family, which was first identified in Africa between 1952 and 1953.[1] It exhibits clinical manifestations such as severe arthritis, as well as back and joint pain, which may persist for weeks or even years.[2] The term "Chikungunya" comes from the Makonde language, signifying "that which bends up," which accurately describes the bent posture of patients afflicted with severe arthralgia.[3] In 1958, the first Asian outbreak occurred in Bangkok, and followed in various Asian countries. In India, the

first CHIKV epidemic was documented in 1963 in Kolkata, West Bengal, killing over 200 people, primarily children.[4] After 32 years, CHIKV emerged and infected 13 states, including about 1.4 million people. Additionally, the outbreak had unexpected clinico-pathological complications such as CHIKV-related deaths and mother-to-child transmission.[5] The global spread of CHIKV may increase the public health risk of Chikungunya. The extensive transmission could increase cases and outbreaks, highlighting the need for effective prevention, supervision, and management.

Chikungunya fever is primarily treated symptomatically, as there are currently no effective vaccines or antivirals available.[6,7] Despite its global health impact, Chikungunya has no specific antiviral treatment. Patient care predominantly focuses on symptomatic treatment, including analgesics, anti-inflammatory drugs, and physiotherapy for joint pain. Despite clinical testing, no vaccine candidates have received regulatory approval for broad usage. The lack of effective treatments underscores the urgent need for the development of antiviral drugs. Medicinal plants and natural items are regarded as the sole method for therapies or proper treatment for various severe illnesses.[8] *C. dactylon*, a popular perennial indigenous grass known as Bermuda grass, occurs naturally and ornamentally in tropical and humid temperate climates.[9,10] Historically, Indian herbalists have used *C. dactylon* as an oral supplement against fevers, diabetes, ulcers, cardiac arrhythmia, diarrhea, gout, rheumatism, and chronic inflammatory conditions. *C. dactylon*, a member of the Poaceae family, is known for its antibacterial, antidiabetic, anti-hyperlipidemia, anti-inflammatory, and antiemetic properties.[11,12] Furthermore, C. dactylon extract has been reported to have promising antiviral activity against shrimp's white spot syndrome virus (WSSV) via *in vitro* and *in vivo* approaches.[13] A previous study demonstrated that apigenin and luteonin ethanolic fractions from *C. dactylon* at 50 µg/mL suppressed 98% of CHIKV activity in Vero cells.[42]

The present research explores the in vitro antiviral potential of an aqueous extract of *C. dactylon* against CHIKV infection. The various chemical compositions of plant extracts may yield synergistic effects superior to those of single-compound methods, resulting in greater therapeutic potential.

MATERIALS AND METHODS

Preparation of Plant Extract

The entire plant (*C. dactylon*) was obtained from the Maharshi Dayanand University campus in Rohtak, India, and authenticated by a university expert. The geographic coordinates of the collection site are latitude 29.670019°N and longitude 75.817738°E. The plant material was cleaned, air-dried, ground into powder, and 20 g was subjected to Soxhlet extraction using double-distilled water as solvent. The extract was filtered through Whatman filter paper No. 1, and the solvent was evaporated using a hot air oven at 45°C to yield a dried, concentrated crude extract, which was then preserved at -20°C until use.

Stock preparation of *C. dactylon*

For *in vitro* studies, a stock solution of *C. dactylon* (100 mg/ml) was prepared in DMSO (50%) and purified by filtration using a syringe filter (0.22 ìm pore size). The working solution was prepared from stock in Minimum Essential Medium (MEM) at different concentrations ranging from 500 to 1.9 µg/mL.

Phytochemical Analysis

Fourier transform infrared (FTIR) spectroscopy

The FTIR is an efficient technique for identifying and characterizing compounds or functional groups present in an unknown plant extract mixture. It is carried out within the range of 4000–400 cm^{-1}. The obtained spectrum data were analyzed against reference graphs to evaluate the functional groups present in the extract.

Gas chromatography-mass spectrometry (GC–MS)

The Shimadzu GC-MS-QP2020 was used to identify volatile organic compounds in a plant extract sample. Helium, a carrier gas (1.0 mL/min flow rate), is used on the SH-Rxi-5Sil MS column (I.D.: 30 m × 0.25 mm; film thickness: 0.25 µm). A 1 ìL volume was used to deliver a splitless infusion. The initial oven temperature was set at 40°C for 2 minutes, then subsequently raised (10°C/min) for 5 minutes, not exceeding 150°C, and then elevated (20°C/min) up to 250°C for 10 minutes. The ion source and interface temperatures were sustained at 230°C and 280°C, respectively. The mass spectrometer was configured to 70 eV in electron impact mode, scanning from 35 to 500 m/z (2000 amu/sec), with a solvent delay of 3 minutes. The

NIST Library's ionization spectra were used to identify the chemicals and to determine their area and proportion in the total composition.

***In vitro* analysis of plant extract**

Cells and Viruses

Vero CCL-81 cells were used and cultured in MEM enriched with FBS (10 %, heat-inactivated) and 1% antimycotic antibiotic solution (Sigma Aldrich, US) under standard conditions (37°C, 5% CO_2). The CHIKV strain was cultured in T-25 tissue culture flasks and subjected to two passages in Vero cells for virus propagation. Cell supernatants were collected after observing 75% CPE, then prepared into aliquots and stored at -80 °C. The viral titers were quantified via the Foci-forming assay.

***In vitro* Cytotoxicity Assays**

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test determines the cell viability.[14,15] Briefly, vero CCL-81 cells (35×10^3 cells/well) were treated with *C. dactylon* extracts (500-3.90 µg/mL) at two-fold serial dilutions in MEM for 48 hours in 5% CO_2 at 37°C. Introduce MTT dye (10 µL; 5mg/mL in PBS) into respective wells and further incubate for 3 hours. Subsequently, the MTT solution was removed, and DMSO (100 µL) was added to dissolve the formazan crystals. Following 30 minutes of constant stirring, the absorbance was measured at 570 nm using a reference filter of 690 nm. The viability percentage was determined in contrast to untreated cells. The highest concentration of the plant extract that did not show cellular toxicity was determined as the Maximum Non-Toxic Dose (MNTD).

Antiviral assay

In order to evaluate the antiviral potential of aqueous *C. dactylon* extracts against Chikungunya virus (0.01 MOI), three methods were employed using Vero cells: pre-treatment (5 hours treatment with plant extract before virus infection), co-treatment (1 hour treatment of plant extract with virus before infection), and post-treatment (cells were infected before extract treatment). A virus control (VC) was maintained for all treatment conditions in untreated cells. Following 48 hours of incubation under each treatment condition, the morphology of cells was microscopically examined for cytopathic effects (CPE). Culture Supernatants were obtained after three freeze-thaw cycles and used to evaluate the virus particle titre via the focus-forming unit assay. All experiments were conducted in triplicate.

Foci-forming unit assay

The virus particles were quantified using the Foci-forming unit assay (FFU), as described by [16,17.] Vero cells (35000 cells/well) were introduced in a 96-well plate and allowed to grow a monolayer. The cells were infected for approximately one hour using a tenfold dilution of the supernatants from the antiviral assay. Following incubation, the washed cells were cultured with 2% MEM containing 1.8% carboxymethyl cellulose and incubated for 24 hours at 37°C. The cells were rinsed with PBS supplemented with Tween 20 detergent, and then fixed with a chilled acetone and methanol solution at a 1:1 ratio. A blocking buffer (1% bovine serum albumin in PBS) was used for 40 minutes at 37 °C to block. After washing, cells were treated with anti-CHIKV monoclonal antibody (MAb ClVE4/D9 clone; 1:300 dilution) for 40 minutes. Following this, anti-mouse IgG HRP conjugate (1:1000) (Sigma-Aldrich, USA) was added, and the cells were incubated for an additional 40 minutes. Following the addition of True-Blue Peroxidase Substrate (LGC Sercare, USA) under dark conditions, the mixture was kept at room temperature for 15 minutes. Once the blue tinge developed, the foci were visualized and counted to determine the virus titer.

Anti-inflammatory Activity

The egg albumin denaturation assay determined the anti-inflammatory activity of *C. dactylon* extract.[18] Prepare a 5 mL reaction mixture including various concentrations of plant extract (100-500 ìg/ml), and the reference drugs (diclofenac sodium), 1 mL solution of bovine serum albumin (1%), and 4.78 mL of PBS (pH 6.4). The mixture was then incubated for 20 minutes at 37°C and subsequently incubated for 30 minutes at 70°C in a water bath to denature the BSA. The reaction mixture was then cooled to room temperature. Measured the absorbance at 660 nm and calculated protein denaturation activity based on the following formula:

$$\% \, denaturation = \left(\frac{Ab\ control - Ab\ sample}{Ab\ control}\right) \times 100$$

Statistical Analysis

GraphPad Prism 10 (GraphPad Inc., USA) was used for the Statistical analyses. Multiple group comparisons were performed using one-way ANOVA, followed by Tukey's post hoc test, whereas two-group comparisons were conducted using Student's t-test. The threshold for statistical significance was established at *p* d 0.05. All data are represented as mean ± SEM.

RESULTS

Our research shows that *C. dactylon* aqueous extract exhibits potent antiviral activity against the Chikungunya virus, particularly displaying high efficacy in post-treatment administration.

Phytochemical Analysis

Fourier transform infrared (FTIR) spectroscopy

The functional group of bioactive compounds of *C. dactylon* aqueous extract was determined using an FTIR spectrophotometer. Eight major peaks were observed for the FTIR analysis of *C. dactylon,* as presented in Figure 1. The IR spectrum showed an absorption band at 3850.36 cm^{-1}, 3739.75 cm^{-1}, 3675.08 cm^{-1}, and 3611.88 cm^{-1} attributed to O-H stretching, 2356.68 cm^{-1} corresponds to Ca O triple bond vibration, 1744.12, and 1694.45 cm^{-1} correspond to C=O stretching, and 1520.05 cm^{-1} represents N-O asymmetric stretch. As represented in Table 1, these absorbance peak values have been correlated to the online standard infrared spectrum.

Gas chromatography-mass spectrometry (GC-MS)

The result of the GC-MS revealed that the plant extracts recorded over 24 compounds, as shown in Figure 2. The bioactive compounds of the aqueous extract of *C. dactylon* were identified through GC/MS analysis and are listed, along with their retention times, area percentages, classes, molecular formulas, and molecular weights, in Table 2. The most abundant compound was hexadecanoic acid, eicosyl ester. According to a study, structural and kinetic investigations of n-hexadecanoic acid, which is responsible for inhibiting phospholipase A2 (an enzyme involved in inflammation), suggest that it has anti-inflammatory potential. This finding supports the conventional use of n-hexadecanoic acid-enriched medicinal oils in the Ayurvedic system of medicine for treating rheumatic conditions.[19]

***In vitro* Antiviral Screening**

Cell Cytotoxicity Assays

Cell viability was evaluated using the MTT test to determine the cytotoxic effects of *C. dactylon* aqueous extract on Vero cells. The results of cell viability percentages are represented in Figure 3. The highest dose of extract that resulted in more than 90% cell viability was used for evaluating antiviral activity. Cell viability exceeded 90% at concentrations d" 62.5 µg/mL, with an estimated IC50 of 420.67 µg/mL. Further investigations used 62.5ìg/mL concentrations, which proved non-toxic to cells. All tests with each concentration of plant extract and cell control were performed in triplicate.

Microscopic morphological screening of cytopathic effects

The antiviral effectiveness of *C. dactylon* extract in Vero cells was evaluated by microscopic morphological screening 48 hours post-infection to determine the CPE. The uninfected control cells formed a confluent monolayer of fibroblastic,

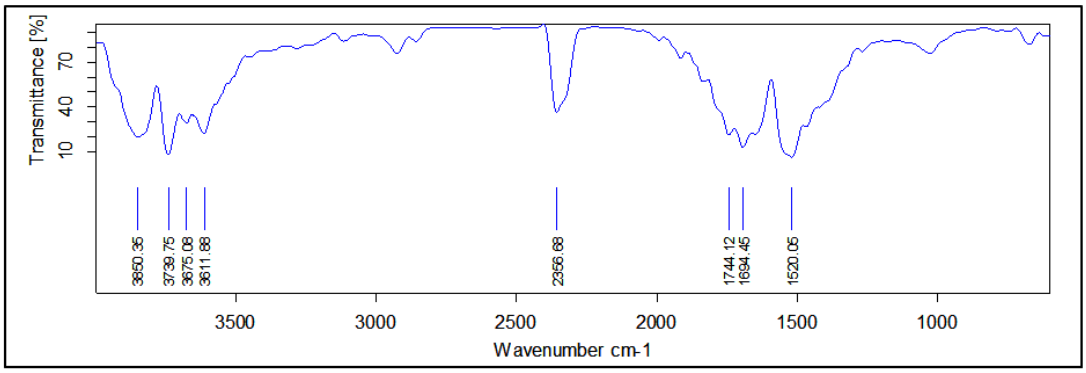

Fig. 1. FTIR spectra of *C. dactylon* dried aqueous extract

elongated, spindle-shaped cells with evident intercellular boundaries. In contrast, virus-infected cells exhibited severe CPE, which resulted in multinucleated syncytia, cell rounding, and substrate detachment, leading to monolayer degradation. The pre-treatment cells exhibited syncytium development and cellular damage similar to those in the virus control. However, co-treatment indicated that the extract provided moderate protection. Post-treatment notably provided substantial cellular protection, maintaining nearly normal fibroblastic shape and inhibiting syncytia development. The extract successfully arrested the Chikungunya-induced cellular damage when supplied post-infection. Figure 4 illustrates the comparative CPE of various treatment conditions of *C. dactylon* extract against cell and viral controls, indicating that post-treatment is more efficient.

Effect of *C. dactylon* extract on CHIKV replication

CHIKV-infected vero cells were treated with MNTD (62.5 ìg/ml) of the extract under pre (prophylactic effect), co (virucidal activity), and post (therapeutic effect) treatment. The results revealed that the extract was effective only under post-treatment conditions at a concentration of 62.5 µg/ml. A graph for anti-CHIKV potential at maximum non-toxic dose under post-treatment condition, compared with the virus control shown in Figure 5. Following the antiviral test, the FFU assay was conducted 48 hours later to determine the virus titer. In the FFU assay, the treatment of the cells 4 hours post-infection with extract at a

Table 1. FTIR spectrum analysis of *C. dactylon* aqueous extract

No.	Wave number	Wave number range (cm^{-1})	Vibration	Functional group
1.	3850.35	4000-3800	O-H stretching and N-H stretching	-
2.	3739.75	3800-3700	O-H stretching	Alcohols and phenols
3.	3675.08	3700-3600	O-H stretching	Alcohols and phenols
4.	3611.88	3700-3600	O-H stretching	Alcohols and phenols
5.	2356.68	3400-2300	Triple bonds (CaO)	-
6.	1744.12	1760-1690	C=O stretching	ä-lactone, esters, cyclopentanone,
7.	1694.45	1700-1600	C=O stretching	Amides, Ketones, Aldehydes, and Carbonyl
8.	1520.05	1650–1580	N–O asymmetric stretching	Nitro Compounds

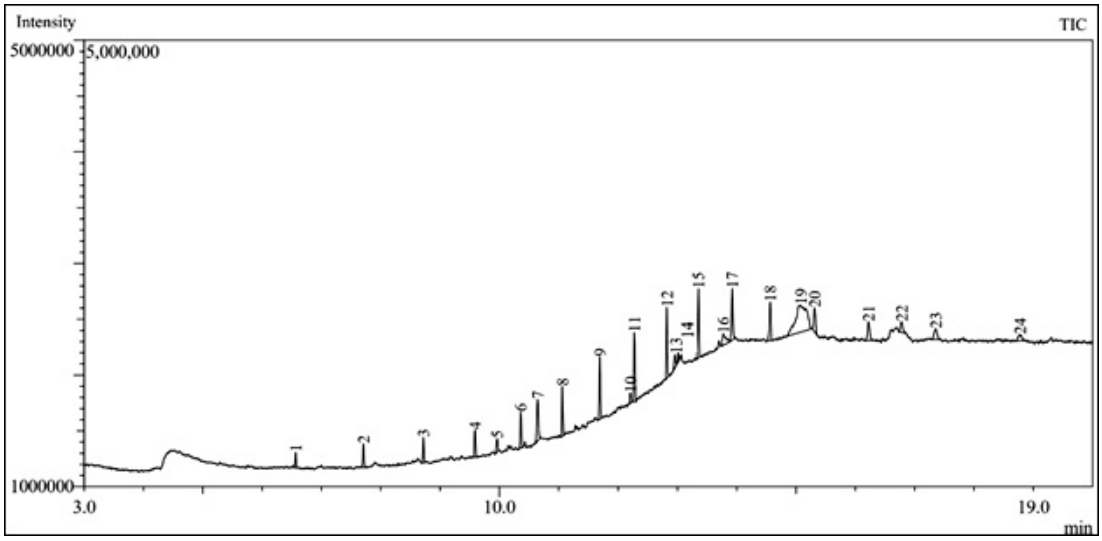

Fig. 2. GC-MS Chromatogram of *C. dactylon* aqueous extract

Table 2. Bioactive compounds found in C. dactylon aqueous extract

Peak#	Retention Time	Area%	Compounds	Class	Molecular formula	Molecular weight (g/mol)
1	6.566	1.35	Cyclohexasiloxane, dodecamethyl-	organosilicon	$C_{12}H_{36}O_6Si_6$	444.92
2	7.709	2.20	Cycloheptasiloxane, tetradecamethyl-	Cyclic Siloxane	$C_{14}H_{42}O_7Si_7$	519.07
3	8.720	2.21	Cyclooctasiloxane, hexadecamethyl-	cyclic siloxane	$C_{16}H_{48}O_8Si_8$	593.2
4	9.589	2.45	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
5	9.963	1.68	Neophytadiene	diterpene	$C_{20}H_{38}$	278.5
6	10.360	2.95	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
7	10.645	4.90	Dibutyl phthalate	benzoic acid esters	$C_{16}H_{22}O_4$	278.34
8	11.061	4.21	Cyclooctasiloxane, hexadecamethyl-	cyclic siloxane	$C_{16}H_{48}O_8Si_8$	593.2
9	11.693	5.37	Cyclononasiloxane, octadecamethyl	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
10	12.207	1.00	Glycidyl palmitate	glycidyl ester	$C_{19}H_{36}O_3$	312.5
11	12.275	5.75	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
12	12.822	6.12	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
13	12.957	1.66	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	fatty alcohols	$C_{17}H_{32}O$	252.4
14	13.011	1.01	Palmitic Acid, TMS derivative	organosilicon	$C_{19}H_{40}O_2Si$	328.6
15	13.354	6.37	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
16	13.773	3.08	9,12-Octadecadienoic acid (Z, Z)-, TMS derivative	linoleic acid	$C_{21}H_{40}O_2Si$	352.6
17	13.923	5.76	Cyclononasiloxane, octadecamethyl-	siloxanes	$C_{18}H_{54}O_9Si_9$	667.4
18	14.563	4.48	Tetracosamethyl-cyclododecasiloxane	organometalloid	$C_{24}H_{72}O_{12}Si_{12}$	889.8
19	15.079	24.64	Hexadecanoic acid, eicosyl ester	wax ester	$C_{36}H_{72}O_2$	537
20	15.314	3.19	Tetracosamethyl-cyclododecasiloxane	organometalloid	$C_{24}H_{72}O_{12}Si_{12}$	889.8
21	16.221	3.43	Tetracosamethyl-cyclododecasiloxane	organometalloid	$C_{24}H_{72}O_{12}Si_{12}$	889.8
22	16.778	1.97	Stigmast-5-en-3-ol, oleate	phytosterol	$C_{47}H_{82}O_2$	679.2
23	17.355	2.52	Tetracosamethyl-cyclododecasiloxane	organometalloid	$C_{24}H_{72}O_{12}Si_{12}$	889.8
24	18.774	1.70	Tetracosamethyl-cyclododecasiloxane	organometalloid	$C_{24}H_{72}O_{12}Si_{12}$	889.8

62.5 ìg/ml concentration resulted in foci reduction from 8.10 (Virus control) to 7.36 Mean $\log_{10}$ FFU/ml (82.25% inhibition in virus titre; p = 0.005).

In Vitro Anti-inflammatory Activity

The anti-inflammatory effects of *C. dactylon* aqueous extract were determined using the egg albumin denaturation assay, performed in triplicate. The extract showed dose-dependent (100-500 ìg/ml) inhibition between 25.90% and 72.38%, with an IC_{50} of 2.4 ìg/ml compared to diclofenac sodium (a standard drug), which exhibited 38.64% to 75.14% inhibition with an IC_{50} of 3.2 ìg/ml, as shown in Figure 6. The results indicate the strong anti-inflammatory potential of the plant extract in contrast to diclofenac, suggesting its therapeutic capability as a natural anti-inflammatory candidate.

DISCUSSION

In recent years, significant viral infections like influenza,[20,21] hepatitis,[22] COVID-19,[23] Zika,[24] Nipah,[25] Marburg,[26] Monkeypox,[27] dengue,[28] Chikungunya, and others have also raised serious public health concerns. Chikungunya fever is a global public health challenge in tropical and subtropical countries. Currently, there are no licensed medications or vaccines effective against Chikungunya. Mosquito management is the primary

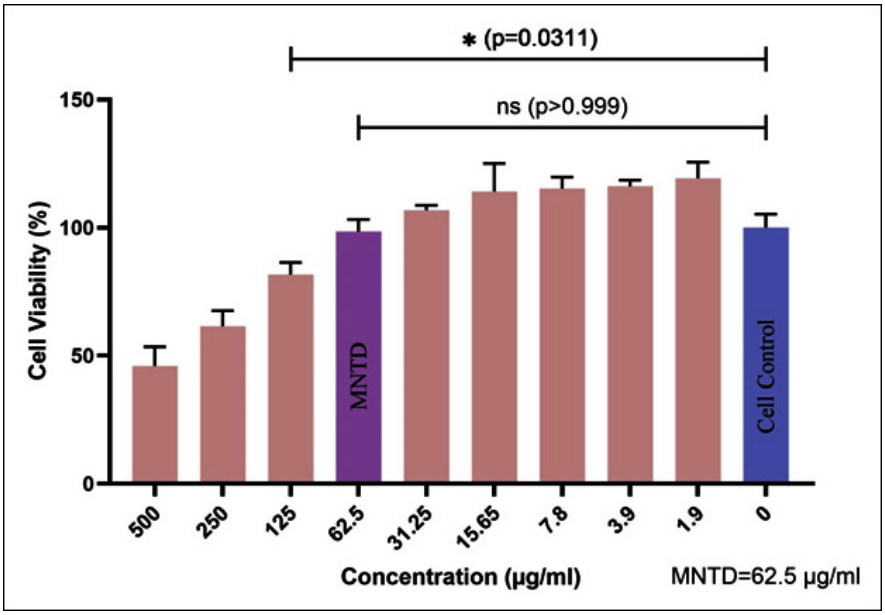

Fig. 3. Percentage of Cell viability at different concentrations of *C. dactylon* aqueous extract using MTT assay

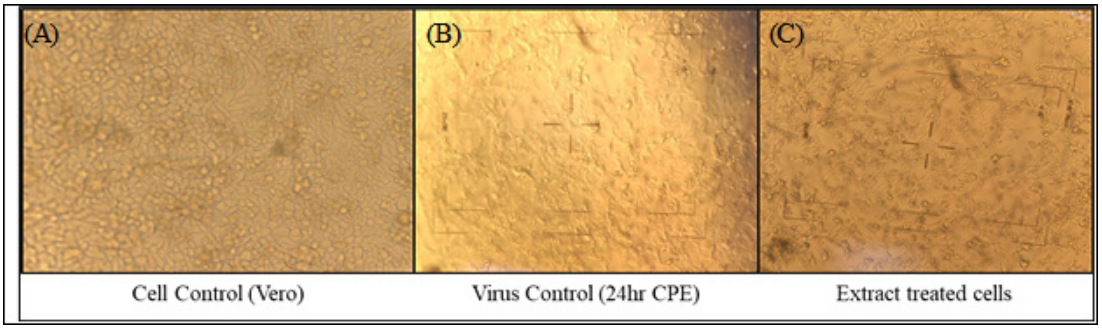

Fig. 4. Post-treatment using Vero cells. (A) Cell Control; (B) Virus control showing CPE; (C) Effect of *C. dactylon* aqueous extract on CHIKV-infected cells

preventive strategy for CHIKV. A wide range of drug candidates has been evaluated for anti-CHIKV activity, and the search for effective therapies is ongoing due to safety concerns associated with the expedited vaccination licensing procedure and potential long-term adverse consequences.[29] Chemically produced antivirals have a wide range of adverse effects, in contrast to traditional therapies.[30] The developments of plant-based medicine are being researched and developed, offering many potential uses and benefits.[31,32] The emergence of viral diseases required the exploration of alternative therapeutic approaches. A broad spectrum of traditional medicinal plants and herbs demonstrating significant antiviral potential against various pathogenic viruses, including influenza,[33,34] dengue,[35,36] Chikungunya,[37,38,39,] and COVID-19.[23,40] Therefore, plant-based anti-Chikungunya medications may function as an alternative therapy for mosquito-transmitted diseases. Several plant extracts exhibit strong antiviral properties against CHIKV, such as silymarin from Silybum marianum, which inhibits viral replication. Additionally, Ipomoea aquatica and Persicaria odorata also inhibit viral replication. Other species, such as *Tinospora cordifolia* and *Andrographis paniculata* prevent genome replication, while *Tradescantia spathacea* and *Rhapis excelsa* demonstrate direct virucidal effects. Additional plants, including *Ocimum tenuiflorum*, *Zingiber officinale*, *Picrorhiza kurroa*, *Terminalia chebula*, *Cedrus deodara* and *Commiphora wightii*

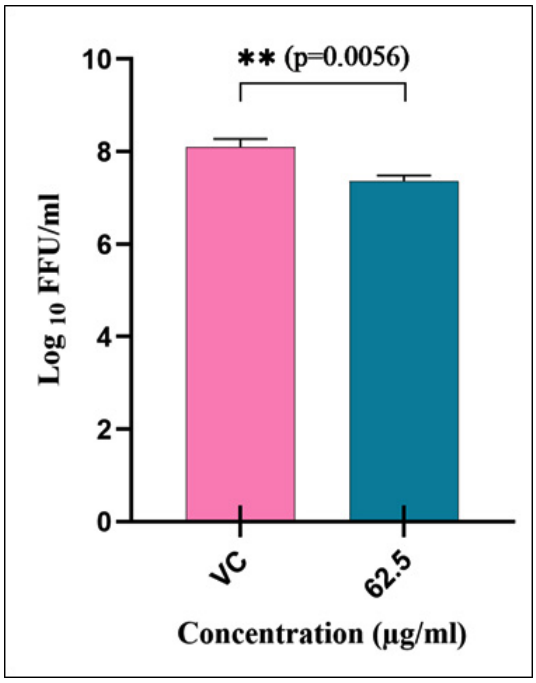

Fig. 5. Antiviral screening of *C. dactylon* aqueous extract against CHIKV at maximum non-toxic concentration under post-treatment conditions, compared with the virus control

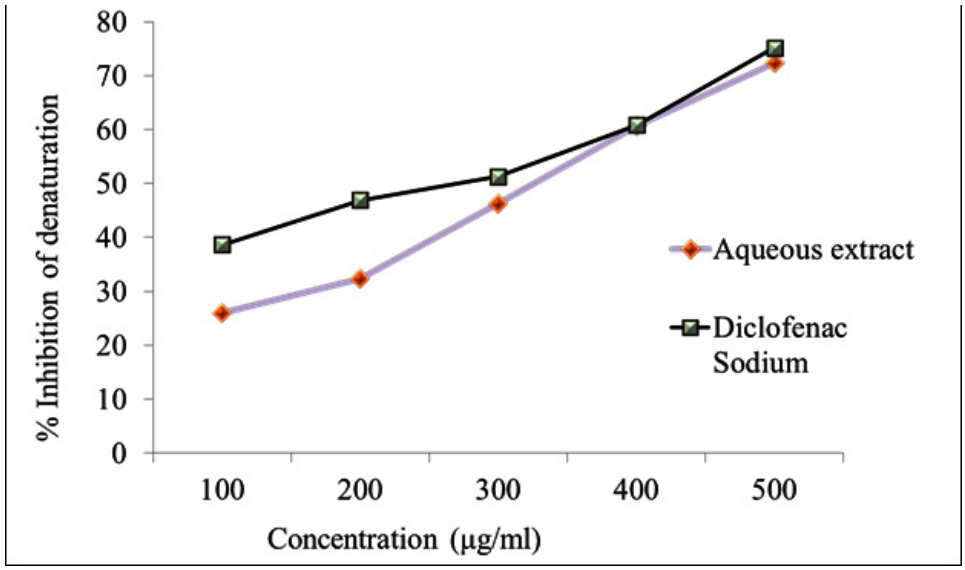

Fig. 6. Percentage inhibition of denaturation by *C. dactylon* extract and diclofenac at different concentrations

also disrupt CHIKV replication or attachment. Key active compounds identified are andrographolides and nobiletin, with the polyherbal formulation Nilavembu kudineer showing significant antiviral potential.[41] Certain isolated flavonoids, such as silymarin, baicalein, nobiletin, quercetagetin, and fisetin, exhibited strong anti-CHIKV action.[42] The ethyl acetate extracts of *Stillingia lineata* having highest efficiency against CHIKV, suggesting its potential as a natural antiviral candidate.[43] According to the Ayurvedic medicine system, *C. dactylon* is employed as a traditional remedy to treat a wide range of health problems, including cough, diarrhea, hemorrhage, bronchitis, dysentery, cramps, epilepsy, measles, piles, dropsy, cystitis, convulsions, calculus, warts, vitiligo, high blood pressure, hysteria, asthma, cancer, tumors, sores, anasarca, rubella, snakebites, carbuncles, stones, urogenital problems, wounds, eye problems, weak eye sight, headache, and dermatitis. It also effectively alleviates pain, inflammation, dental discomfort, and influenza in youngsters. The astringent properties of the extracted plant juice are used to stop bleeding from cuts and wounds. A combination of leaf paste and honey is beneficial against nosebleeds. Doses of plant extract mixed with honey, taken two to three times a day, can effectively treat heavy menstrual bleeding. Acute vaginal hemorrhage is alleviated by applying a thin layer of a plant extract-based paste to the lower abdomen. A combination of *C. dactylon* and sugar works well in the treatment of urine retention.[44]

In this research, we examined the anti-CHIKV potential of the aqueous extract of *C. dactylon* to identify CHIKV targets and pathways for developing novel traditional antivirals. Numerous phytochemicals are responsible for its antiviral action and other therapeutic qualities.[45] Phytochemical research using FTIR spectroscopy revealed that the diverse bioactive chemicals, including phenols, alkaloids, flavonoids, carboxylic acids, and other organic compounds, may be responsible for its biological action. The GC-MS chromatogram displays 24 peaks. The most-abundant compound identified in the GC-MS analysis was hexadecanoic acid eicosyl ester (24.64%). It exhibits a significant anti-inflammatory property and is used in Ayurvedic medicine to treat rheumatic conditions.[19]

The in vitro anti-chikungunya activity of *C. dactylon* plant extract was investigated using Vero cells. To evaluate its potential adverse effects, it is necessary to conduct an in vitro evaluation of the maximum non-toxic dose of the aquatic medicinal plant extract before its therapeutic use. Aqueous medicinal plant extract displays a cytopathic effect on Vero cells. The MTT assay demonstrated the MNTD of *C. dactylon* aqueous extract, which is non-toxic to Vero cells, showing cell viability above 90% at concentrations up to 62.5 µg/mL, with an estimated IC50 value of 420.67 µg/mL. The concentration of DMSO in the working solution of plant extract was less than 1%, which is considered safe for the cells and does not cause cytotoxicity.[46]

The three different antiviral approaches showed different cytopathic effects on Vero cells. The extract's protective properties were demonstrated through a microscopic morphological examination of CPE. The co-treatment and pre-treatment showed minimal protection to Vero cells. The post-treatment with *C. dactylon* extract effectively prevented the formation of multinucleated syncytia and preserved the nearly normal fibroblastic morphology in virus-infected control cells. This pattern indicates that the extract primarily disrupts active viral replication rather than providing prophylactic protection, highlighting its therapeutic potential. The quantitative antiviral results, which correlate with the cellular protection, further confirm the extract's potential for medical use. The antiviral results from post-treatment were further confirmed by the FFU assay, which measures functional infectious virus titre. Post-treatment has the most effective antiviral action (82.25% reduction in FFU titre). The anti-inflammatory activity is assessed using the egg albumin denaturation assay. The extract exhibited 72.38% inhibition at 500 ìg/mL, with an IC50 of 2.4 ìg/mL, compared to diclofenac sodium (75.14%; IC50: 3.2 ìg/mL). Chikungunya induces debilitating arthralgia due to viral replication and substantial inflammatory reactions; hence, this antiviral and anti-inflammatory combination is necessary for treatment.

Combining antiviral and anti-inflammatory activities in a single extract signifies a therapeutic benefit. By targeting both viral replication and its inflammatory responses, *C. dactylon* extract may provide a comprehensive treatment approach

against Chikungunya fever. This dual-action mechanism is consistent with the traditional use of medicinal plants, which often contain multiple bioactive compounds that work together to act as antiviral agents against complex viral diseases.

CONCLUSION

Chikungunya is a mosquito-borne disease that causes severe joint pain and inflammation, resulting in long-term disability. There are currently no widely approved antiviral drugs or vaccines available for the effective treatment of CHIKV infection. Patient management is largely symptomatic, relying on general analgesics, anti-inflammatory medications, and supportive care to relieve joint pain and fever. The current research highlights the antiviral and anti-inflammatory effects of *C. dactylon* aqueous extract against the chikungunya virus (CHIKV) and its potential uses in medicine. The ability of extract to reduce virus titer by 82% and its anti-inflammatory activity make it a promising candidate for developing natural antiviral therapies. Hexadecanoic acid helps alleviate rheumatological symptoms by reducing the severity of inflammation associated with CHIKV. These findings could have a significant impact on the management of Chikungunya fever and other viral diseases, particularly in areas where these infections are endemic and medical resources are limited.

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This research did not involve any studies with human participants or animals performed by any authors.

Informed Consent Statement

This study did not involve human participants, so informed consent was not required.

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This research does not involve any clinical trials.

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Not Applicable

Authors Contributions

Kumari Soniya: Drafted the manuscript and experimental work; Sanjit Boora, Suman Yadav, and Manisha Sharma are drafting and reviewing the manuscript; Poonam Patil, Abhishekh Kumar Bharti: protocol drafting, data collection, and analysis; Kalichamy Alagarasu, Deepti Parashar, and Samander Kaushik: Conceptualization and manuscript drafting.

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