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ORIGINAL ARTICLE

In-vitro and in silico activity of *Cyamopsis tetragonoloba* (Gaur) L. supercritical extract against the dengue-2 virus

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Abstract Our health and wealth are highly influenced by a number of viruses. Dengue is one of them having a global influence in absence of vaccines and antiviral. WHO suggested that the morbidity of dengue is increasing more than 6 times from 0.5 million in 2010 to over 3.34 million in 2016, following a sharp increase in 2019. The aim of the present study is to check the in vitro and in silico anti-dengue activity of *Cyamopsis tetragonoloba* supercritical extract in cell lines. The optimum yield of supercritical extract was obtained 0.13 g/10 g (1.3% w/w) at 40 °C temp and 15 MPa pressure and further characterized by GC–MS. The antiviral assay was performed on C6/36 cell lines with 100 copies of dengue-2 virus and maximum non-toxic dose (31.25 µg/ml) of supercritical extract and their effect was detected by real-time RT-PCR. This study revealed that *C. tetragonoloba* supercritical extract inhibited the dengue-2 virus (99.9%). GC–MS analysis of *C. tetragonoloba* supercritical extract showed the presence of 10 compounds. The major compounds identified were Hexadecanoic acid, 15-methyl–methyl ester (24.498%); 9,12-octadecadienoyl chloride, (*z*,*z*)- (23.718%); methyl dodecanoic acid (13.228%); methyl-stearate (8.696%); Tridecanoic acid, 12-methyl-, methyl-ester (8.426%), dodecanoic acid (6.102%). The study reveals that *C. tetragonoloba* can be exploited to develop an effective, inexpensive, and specific anti-dengue. The molecular docking study demonstrated the binding energy of 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (− 4.1 kcal/mol), 9,12-octadecadienoyl chloride (*z*,*z*) (− 4.0 kcal/mol) ligands were higher than others. It is concluded that *C. tetragonoloba* can play a major role to inhibit dengue-2 virus.

Keywords Dengue virus · *C. tetragonoloba* · Supercritical extract · GC–MS · Molecular docking

Introduction

Dengue is a very notorious mosquito-borne viral infection, belongs to the family *Flaviviridae*, which contains at least seventy-two viruses including; Zika, Yellow fever, Japanese encephalitis, and West Nile viruses. Dengue is an enveloped, single-stranded, positive-sense RNA virus, transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. There are four serotypes of dengue virus circulating in various parts of the World. Dengue is very dangerous since its origin but its prevalence has increased significantly within the last two decades. Although there is no specific surveillance, World Health Organization (WHO) suggested that more than 100 countries from Asia, Africa and some parts of Europe, South and North America are affected by dengue virus [38]. The number of dengue cases reported to WHO increased by $\sim$ 6 times from $<$ 0.5 million in 2010 to over 3.34 million in 2016, following a sharp increase in 2019 [3]. At present, there is no effective treatment for dengue. Medicinal plants area source for the treatment of common to life-threatening diseases since civilization. Herbal medicine can be alone used for treatment or increase the therapeutics potential

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when used along with conventional antiviral medicines [13]. Many herbs are being routinely used against DENV infection as an alternative approach to reduce disease symptoms [11]. The discovery of natural drugs will be more universal, modified, and involves sensible use of modern and ancient skills of therapeutics in a corresponding way so that the patient and community get the maximum benefits [23]. The present study is focused on *Cyamopsis tetragonoloba* (L.) Taub (guar), which is commonly known as cluster bean belongs to the family *Fabaceae*. It is a small (2-4 feet), drought-resistant legume plant cultivated in semi-arid regions of India and Pakistan. The plant seeds are rich sources of their nutritive values viz vitamins, amino acids, flavonoids and phenolic compounds [18]. *C. tetragonoloba* used as an herbal medicine for dyspepsia, anorexia, digestive aid, cure inflammation, sprains, arthritis, anti-secretory, and anti-oxidant and antibacterial [15]. Guar gum is an herbal medicine that is used for treating cancer and diabetes which is also used as a dietary supplement. It has properties as anti-microbial, anti-tumor, and anti-inflammatory. The leaves of gaur used in asthma, and to treat night blindness, constipation, cough, and Vata [36]. Several secondary compounds were present in gaur seeds and works have been carried out [1, 6]. The present study aims to analyse the in vitro and in silico anti-dengue potential of *C. tetragonoloba* supercritical extract (SFE) in animal cell culture model and to analyse the secondary compound in the extract by gas chromatography–mass spectrometry (GC–MS).

Materials and methods

Chemicals and reagents

An in vitro study was done in C6/36 cell with the help of minimum essential medium (MEM, Batch No. 0000319279), streptomycin sulphate (100 µg/ml, Hi-Media, Batch No. 0000187551), Penicillin (100 U/ml, Sigma-Aldrich Batch No. BCBN 3112V), Trypsin (Hi-Media Batch No. 0000285329) and Fetal Calf Serum (FCS, Gibco NV, USA, Batch No. 1584260). Cell viability/cytotoxicity was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Hi-Media, Batch No. 0000263610). Analysis of anti-dengue assay was done using the commercially Geno-Sen's Dengue S1-S4 PCR kit that contains a buffer, virus standard, enzymes, dNTPs, dengue specific primer, and probes.

Collection of plants materials

C. tetragonoloba was selected on the basis of traditional knowledge and collected from agriculture fields of village Majra (Dubaldhan), district Jhajjar, Haryana, India. The geographical location of the collection site is Latitude 28° 41′ 15.432″ N and Longitude 76° 52′ 47.028″ E. Plant samples were transported to the laboratory in sterile bags. After proper identification by a taxonomist, the plant material was washed with tap water to remove the dust and rinse with distilled water. The material was stored in a shade and dry place at room temperature and minced after dry.

Preparation of supercritical fluid extraction of *C. tetragonoloba*

C. tetragonoloba extract was prepared by using Supercritical Fluid Extraction Machine (Applied Separation Inc. U.S.). Ten grams of the plant powder was loaded into the stainless steel extraction cell of the machine. The CO_2 used as a solvent in SFE. The flow rate was maintained at 1.6 ml/min with static-dynamic mode (1 h static and 30 min dynamic mode). The method of extraction of the plant was standardized and optimized at 40 °C temperature and 150 bar pressure. The obtained SFE extract was lyophilized, weighed and stored at 4 °C for further use.

Determination of cell viability by MTT assays

The maximum non-toxic dose (MNTD) of *C. tetragonoloba* supercritical fluid extracts was determined on 90% confluent C6/36 cells. Approximate, 1×10^5 cells/well were seeded into 96-well flat bottom plate (Nunc, Thermo Fisher Scientific, and the USA) and incubated at 28 °C in a 5% CO_2 incubator overnight. After 90% confluency of C6/36 cells, the medium was removed and the cells were treated with a serial dilution of different concentrations (ranging from 1000 µg/ml to 15.62 µg/ml) of *C. tetragonoloba* supercritical fluid extracts in triplicates. In the negative control, 100 µl of the growth medium was used. After incubation of 96 h, the medium of each well was replaced with 10 µl of MTT, 3-(4,5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromidesalt solution (5 mg/1 ml in PBS, as stock) and again incubated for 3 h at 28 °C in 5% CO_2 incubator. MTT solution was discarded without disturbing the cells and 100 µl of DMSO was added into each well to stop the reaction. The 96 wells plate was continuously shaken until all formazan crystals dissolved. Cell viability was determined with the help of absorbance values obtained by using a microplate reader (Bio-Rad, USA) at 595 nm.

Cell culture and virus isolation

The Aedes cell line C6/36 (ATCC® CRL-1660 TM) was maintained at 28 °C in 5% CO_2 incubator with the help of

minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 2 mML-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml). A total of 500 µl of an appropriate dilution of dengue-2 standard strain was inoculated onto confluent C6/36 cells in 25 cm^2 tissue culture flask and then incubated at 28 °C incubator with 5% CO_2. After 45 min, the virus growth media was added and the cells were further incubated. Even after proper incubation, no cytopathic effect or no plaque formation takes place. Then, flasks stored in the deep freeze and the lysates were harvested and viral RNA was extracted by using a commercial QIAmp Viral RNA mini kit (Qiagen, Germany) according to the manufacturer's protocol. The viral titer of the dengue-2 virus was determined in cell culture lysate by quantitative real-time RT-PCR (ABI-7500 Real-time) along with known standards of dengue, which contains 10^1–10^5 copies/ml (S1–S5) provided by Geno-Sen's Dengue 1–4 commercial kit. A calibration curve was plotted with positive control. The 100 copies/ml of dengue-2 viruses were determined form the lysate, for further anti-viral experiments in the present study.

In vitro anti-dengue assay

The anti-viral assay was initiated by incubating 96 wells plate having a monolayer of C6/36 cells. The simultaneous mode of treatment was carried out to perform the anti-viral assay. The 100 µl of viral suspension of DENV-2 (100 copies/wells) was treated with an equal volume of the non-toxic dose (31.25 µg/ml) of *C. tetragonoloba* plant extract was inoculated in 96 well plates. The plate setup included a cell control, a virus control (cells with back titration of 10, 100 and 1000 copies of dengue-2 virus) and cell viability control (cell with MNTD of *C. tetragonoloba* extracts) as well as experimental well (cell with 100 copies of the dengue-2 virus treated by MNTD of *C. tetragonoloba* extracts) in 96 replica plate. After that, the plate was sealed with parafilm and incubated on ice for 45 min and shaken gently after every 10 min. The mixture from each well of the replica plate was transferred into respective wells of the experimental plate without disturbing the C6/36 cells layer of experimental plate. After inoculation, the experimental plate was sealed with parafilm and incubates for 45 min at 28 °C in the CO_2 incubator. The medium was aspirated from the experimental plate wells. Then, the virus growth medium (VGM) was added without disturbing the cells layer in all wells (including cell control wells). The experiments were carried out in triplicates. Further, the 96-well plate was incubated at 28 °C in a CO_2 incubator for 7 days. After proper incubation, the plate was frozen at − 70 °C and the lysates were harvested and RNA was extracted from each aliquot and quantifies the virus to study the antiviral effect of *C. tetragonoloba* extracts.

Anti-viral assay by qRT-PCR

TaqMan probe chemistry-based Real-time RT-PCR was performed to determine the potential of *C. tetragonoloba* on DENV-2. The commercially available Geno-Sen's Dengue S1-S4 PCR kit was used to perform the antiviral assay on ABI 7500 real-time PCR instruments. The commercially kit contains a specific master mix for specific amplification and quantification of dengue viruses. All the PCR reagents were thawed. The master mix was prepared following the kit manufacturer's instructions. After that, PCR tubes were prepared by adding 10 µl master mix and 15 µl of extracted RNA from each lysate from cell/negative control, virus/positive control, experimental/dengue-2 virus treated by MNTD of *C. tetragonoloba* extracts, and standards (Dengue S 1-5). Then, all the reagents were mixed properly by pipetting up and down in the PCR tubes. Then the amplification tubes were closed and transferred into the ABI 7500 real-time thermocycler. The reaction conditions of PCR were as follows: reverse transcription at 50 °C for 15 min, denaturation at 95 °C for 10 min, as followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s and a final extension step at 72 °C for 15 s. The fluorescence emission data were collected during the annealing step.

Data Analysis

The percentages of cell viability of supercritical plants extract concentrations and anti-dengue data was analyzed by Microsoft Excel 2007 with the help of Tukey's test (each treatment mean value different from each other and compared to control). The results were expressed as mean ± standard deviations. The cell viability of plant was calculated as:

$$\text{Cell viability}\,(\%) = \frac{\text{Absorbance treated cell} - \text{Absorbance blank}}{\text{Absorbance cells control} - \text{Absorbance blank}} \times 100$$

Phytochemical analysis using gas chromatography–mass spectrometry (GC–MS)

Phytochemical analysis of *C. tetragonoloba* SFE extract was carried out using GC–MS analyser (BRUKER SCION 436-GCSQ). Helium (99.9%) was used as carrier gas at a flow rate of 1.5 ml/min in split mode. The RESTEK Rtx®-5(cross bond® 5% diphenyl/95% dimethyl polysiloxane) column of suitable dimensions was used. The extract was

dissolved in methanol solvent (HPLC grade) and filtered through Whatman filter paper 0.22 µm. 1µL of the plant sample was injected into the column with an inlet temperature of 280 °C. The conditions were initially programmed at temperature 72 °C hold for 2.5 min and then further amplified at a rate of 10 °C per minute until reached up to 320 °C; hold for 1 min. Total running time was 23.7 min including 3 min solvent delay. The mass spectrum of compounds present in the extract was obtained by electron ionization at 70 eV and the detector operates in scan mode 30 to 500 Da atomic units. The ionization spectra were matched with the NIST (National Institute of Standards Technology) library to identify the compounds along with their area and percentage of the total.

Preparation of ligands and protein structure

The non-structural proteins of DENV-2, NS1 (PDB IDs: 4O6B) were downloaded from PDB (protein data bank) because of better resolution and used a target for docking. The 3D structures of plant ligands were downloaded from PubChem. Ten ligands were selected for docking were Methyl dodecanoic acid (PubChem ID:5282687), Dodecanoic acid, (PubChem ID:3893), tridecanoic acid 12-methyl-, methyl-ester (PubChem ID: 21204), 3,7,11,15-tetramethyl-2 hexadecen-1-ol (PubChem ID: 5366244), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (PubChem ID: 6782), Hexadecanoic acid, 15-methyl-, methyl ester (PubChem ID: 522345) 9,12-octadecadienoyl chloride, (*z*,*z*)-, (PubChem ID:9817754), methyl stearate, (PubChem ID: 8201), tritetracontane (PubChem ID: 522398), tetracontane, 3,5,24-trimethyl (PubChem ID: 41344). The binding orientation and affinity of the ligand to target was predicted by 1-Click docking tool.

Results

Optimization of supercritical condition for extraction

The maximum yield (1.3%) of a supercritical extract of *C. tetragonoloba* plant was obtained at 40 °C temperature and 15 MPa pressure of the SFE extractor machine.

Maximum non-toxic dose (MNTD) of plants

The 31.25 µg/ml was maximum non- toxic dose of *C. tetragonoloba* found, when cell viability assay was performed with the C6/36 cell-line. The MTT assay was versatile and to determine the cell viability and cytotoxicity of the cells. The result of cell viability has been shown in Fig. 1.

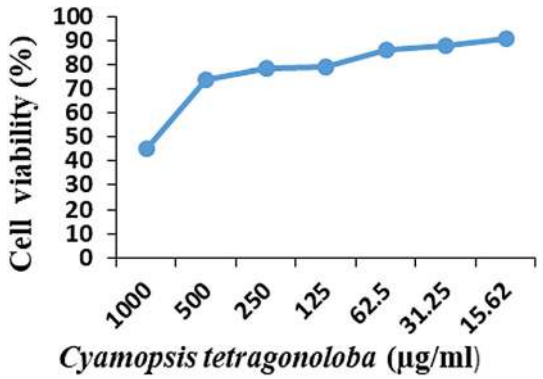

Fig. 1 Maximum non- toxic dose of *C. tetragonoloba*

Quantitative antiviral assay

This study revealed that the *C. tetragonoloba* supercritical extract inhibited the 99.9% dengue-2 virus. The percentage of reduction in viral load of an active extract was compared with the positive control containing the same copy number of dengue-2 viruses, which had 1.76×10^6 copies/ml of the virus after 7 days post-infection in the absence of any extract. The amplification curve was plotted with back titration which contains S1-1000 copies, 10 copies, 100 copies with infected plant extract. The *C. tetragonoloba* CT value was compared to virus control CT value (18) which contains 100 copies. If CT value increases then the virus decrease. An amplification curve depicting the anti-dengue activity of plant *C. tetragonoloba* has been shown in Fig. 2.

Phytochemical analysis using GC–MS

Organic compounds of *C. tetragonoloba* were identified by GC–MS analysis and spectra result was matched with the National Institute of Standards Technology (NIST) MS

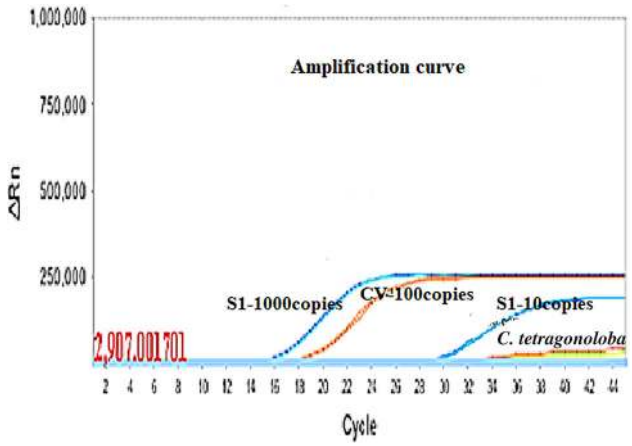

Fig. 2 Amplification inhibition curve of the dengue-2 virus by *C. tetragonoloba* SFE extract (S1- Positive control)

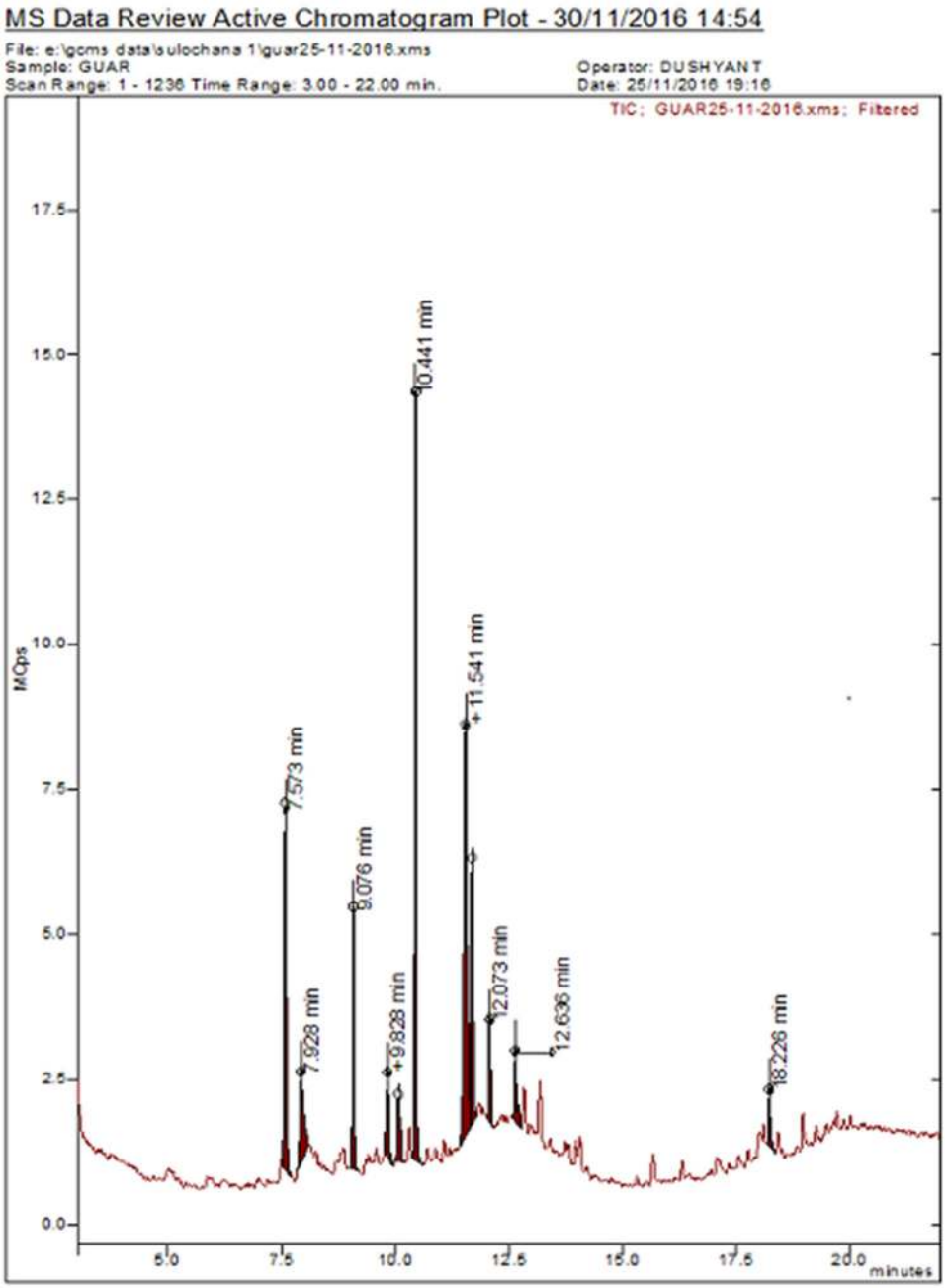

Fig. 3 GC-MS of *C. tetragonoloba* SFE extract

library in GC–MS. GC–MS analysis of *C. tetragonoloba* SFE extract showed the presence of 10 compounds when compared with the NIST database (Fig. 3 and Table 1). The major compounds identified were Hexadecanoic acid, 15-methyl–methyl ester (24.498%); 9,12-Octadecadienoyl chloride, (*z*,*z*)- (23.718); methyl dodecanoic acid (13.228%); tridecanoic acid, 12-methyl-, methyl stearate (8.696%); Tridecanoic acid, 12-methyl-, methyl-ester (8.426%), Dodecanoic acid (6.102%).

Molecular docking

Ligand shown with blue color (ball and stick) and NS1 with grey color (Ribbon). The binding affinity is shown in bracket along with the name of respective compound and interacting residues illustration between protein (yellow and green) and ligand (white). The hydrogen bonding interaction between the ligands and protein was given in Fig. 4. The higher binding energy obtained from the

Table 1 Components detected in *C. tetragonoloba* bean SFE extract

S. No	RT	Name of compounds	% Area	Class of compound	Formula	MW
1	7.571	Methyl dodecanoic acid	13.228	Fatty acid	$C_{13}H_{26}O_2$	214
2	7.925	Dodecanoic acid	6.102	Fatty acid	$C_{12}H_{24}O_2$	200
3	9.079	Tridecanoic acid, 12-methyl-, methyl-ester	8.426	Fatty acid	$C_{15}H_{30}O_2$	242
4	9.833	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	3.400	Terpene alcohol	$C_{20}H_{40}O$	296
5	10.064	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	3.364	Fatty acid	$C_{16}H_{22}O_4$	278
6	10.448	Hexadecanoic acid, 15-methyl-, methyl ester	24.498	Fatty acid	$C_{18}H_{36}O_2$	284
7	11.541	9,12-Octadecadienoyl chloride, (*z*,*z*)-	23.718	Fatty acid	$C_{18}H_{31}$ CIO	298
8	11.679	Methyl stearate	8.696	Fatty acid	$C_{19}H_{38}O_2$	298
9	12.079	Tritetracontane	3.420	Fatty acid	$C_{43}H_{88}$	605
10	12.633	Tetracontane, 3,5,24-trimethyl-	3.085	Alkane	$C_{43}H_{88}$	605

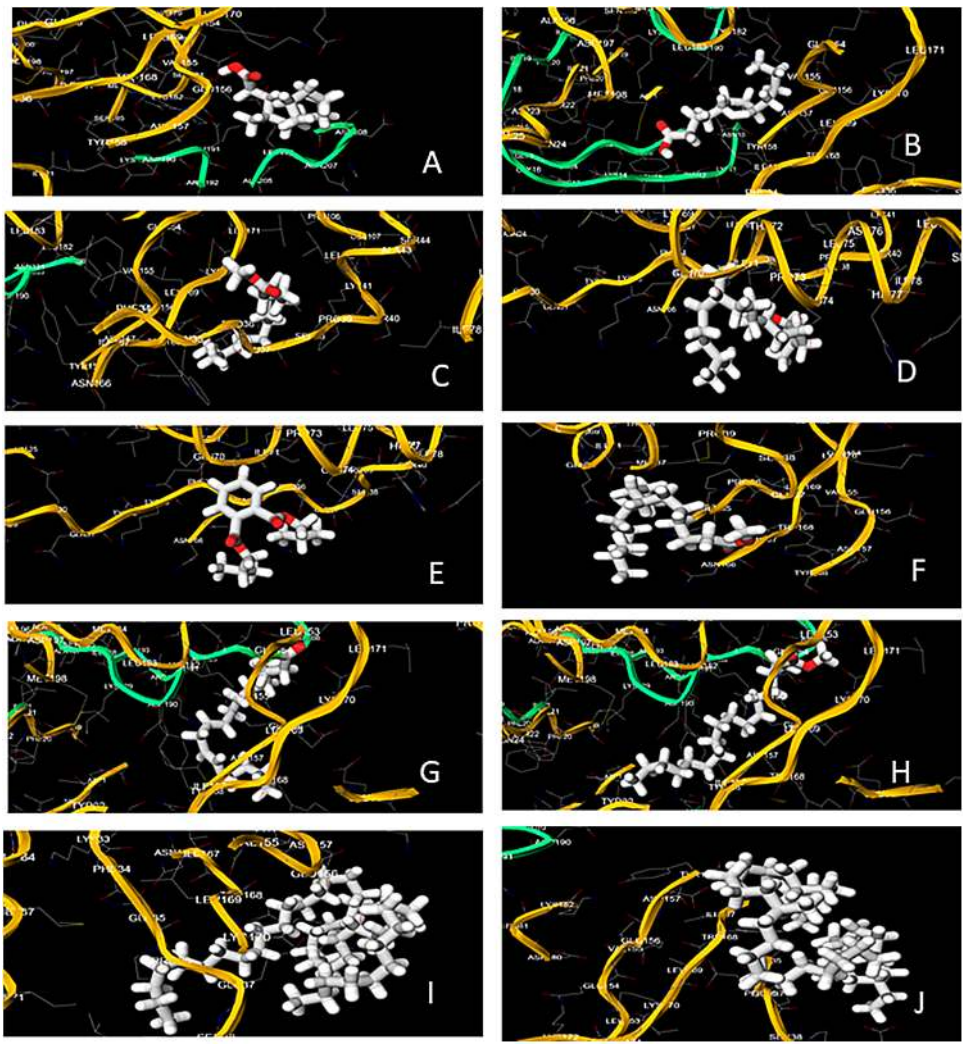

Fig. 4 Hydrogen bonding interaction of dengue NS1 protein with ligands. **a** Methyl dodecanoic acid, **b** dodecanoic acid, **c** tridecanoic acid, 12-methyl-, methyl-ester, **d** 0.3,7,11,15-tetramethyl-2 hexadecen-1-ol, **e** 0.1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, **f** hexadecanoic acid, 15-methyl-, methyl ester, **g** 0.9,12-octadecadienoyl chloride, (*z*,*z*)-, **h** methyl stearate, **i** Tritetracontane, **j** tetracontane,3,5,24-trimethyl-

ligands at decreasing order i.e. 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (− 4.1 kcal/mol), 9,12-Octadecadienoyl chloride (*z*,*z*) (− 4.0 kcal/mol), dodecanoic acid, (− 3.9 kcal/mol), tridecanoic acid, 12-methyl-, methyl-ester (− 3.8 kcal/mol), 3,7,11,15-tetramethyl-2 hexadecen-1-ol (− 3.8 kcal/mol), methyl

dodecanoic acid (− 3.6 kcal/mol), methyl stearate (− 3.0 kcal/mol), Hexadecanoic acid, 15-methyl-, methyl ester (− 3.0 kcal/mol), tetracontane, 3,5,24-trimethyl (− 2.5 kcal/mol), and Tritetracontane (− 2.3 kcal/mol).

Discussion

The viral outbreaks like Crimean Congo hemorrhagic fever, Ebola, Lassa fever, Marburg virus, SARS- CoV, MERS-CoV, Nipah, Zika virus, Rift Valley fever virus and now COVID-19 are some evidence of our disaster. Nipah virus, Zika virus, and COVID-19 outbreaks in India are a few episodes [17, 19, 32, 34]. For the effective control of a viral outbreak, there should be a rapid, sensitive, specific and cost-effective diagnosis assay should be present. Molecular diagnosis assays are the best diagnostic methods as compared with other methods [2, 4, 7, 8, 31]. Dengue is still a big challenge for most of the countries and has become a global health issue. There are no commercial anti-viral drugs available in the market against it. Medicinal plants or their derivatives are of a crucial therapeutic aid for various diseases [20, 26, 37]. Drugs such as morphine, quinine, quinidine, aspirin, colchicines, digoxin, artemisinin, taxol, tubocurarine, ephedrine, vincristine, and vinblastine have been derived from several medicinal plants are used in the treatment of the different type of diseases. Therefore, research on medicinal plant extracts and their bioactive compounds which could be safer and cheaper than synthetic drugs is gaining importance. Many other studies have demonstrated the value of medicinal plants against dengue serotypes [16, 21, 28].

In the present study, we demonstrated that the supercritical extracts of *C. tetragonoloba* show highly significant anti-viral activity against the dengue-2 virus quantitatively with the help of a real-time PCR assay. Supercritical fluid extraction (SFE) is a new extraction technique that is more accurate, safe, reliable, and time-saving, with a higher yield and better-purified compounds compared to conventional methods. The recently reported TaqMan amplification system works the one-step RT-PCR protocol and is a sensitive method for quantification. A previous study suggested that the MNTD of methanolic extracts of *A. paniculata* and *O. sanctum* are 0.050 and 0.100 mg/ml have anti-dengue activities against DENV-1 serotype on the Vero E6 cells [35]. In another study reported the MNTD of methanolic extracts of *A. paniculata* and *O. sanctum* as 0.020 mg/ml and 0.023 mg/ml are effective against DENV-1 on HepG2 cells [22]. In the present study, the MNTD of *C. tetragonoloba* extract was found 31.25 µg/ml effective against the DENV-2 virus on C6/36 cells. A study demonstrated that silver nanoparticles of *Andrographis paniculata, Phyllanthus niruri* and *Tinospora cordifolia* showed potent inhibitory activity against the chikungunya virus [33]. For the antiviral assay, a real-time PCR was performed using either fluorescent hydrolysis (TaqMan) probes, as the TaqMan based RT-PCR assay is more specific than an SYBR green-based RT-PCR assay [29].

C. tetragonoloba bean contains common chemical components e.g. fatty acids, and amino acid. Beans of gaur contain high contents of fats, proteins and carbohydrates. A study demonstrated that the bean of gaur had a high percentage of linoleic acid and the ethanolic extract of C. *tetragonoloba* bean showed antioxidant and anticancer activity. Gaur plant has been shown to be an effective the hypocholesterolemic agent [5, 24]. The *C. tetragonoloba* showed an anti-diabetic effect, anticoagulant activity, anti-ulcer, hemolytic activity, anti-asthmatic activity, anti-inflammatory activity, antibacterial, hypoglycemic and hypolipidemic effect [10, 12, 25, 27, 30]. In the present study, the supercritical extract of gaur contain Hexadecanoic acid, 15-methyl-, methyl ester (24.498%), 9,12-octadecadienoyl chloride, (*z*,*z*)- (23.718), Dodecanoic acid, methyl (13.228%), are the major compounds found in SFE extract of *C. tetragonoloba*. In previous study, the aqueous and methanol extract of *C. tetragonoloba* contains major constituents that were more inositol and 7-tetradecenal, (Z), stigmasterol, 10,12-hexadecadien-1-ol and 9,12-octadecadienoyl chloride, (*z*,*z*) and have reported antioxidant, anti-cancerous and thyroid inhibiting properties [14]. Further research on this may help in the development of medicines against the dengue virus. Molecular docking in silico method is used in drug development. In this method, phytochemicals are matched with viral targets to find interactions between the drug and disease-producing agents by using the computational method. NS1 are essential for viral replication. NS1 is the only protein that is continuously secreted by infected host cells. NS1 is found in multifunctional in nature i.e. production of infectious virus particles, by using an innovative trans-complementation system with fully functional epitope-tagged in NS1 protein, also interacts with the structural proteins, etc. Due to multifunctional in nature, NS1 protein appears as an attractive target for antiviral therapy [9]. The researchers have put great effort in search of effective molecules for targeting different structural or non-structural proteins of the dengue virus.

In conclusion, supercritical extracts of plants were used to inhibit dengue virus. The inhibition profile of *C. tetragonoloba* plant extracts for dengue-2 showed significant anti-dengue activity. The GC–MS identified natural compounds may be responsible for their inhibitory activity against dengue-2. At a result of molecular docking and in vitro study demonstrated that the *C. tetragonoloba* plant extract machanism of action is to cease the dengue replication (NS1). As anti-dengue activity is associated with *C.*

tetragonoloba further research is required. Plant extracts and its derivatives have the potential to develop newer anti-dengue drugs with unique drug targets.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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