Therapeutic Potential of Medicinal Plants against Dengue Infection: A Mechanistic Viewpoint

Mohammad Altamish, Muzayyana Khan, Mirza Sarwar Baig, Bharti Pathak, Veena Rani, Jamal Akhtar, A. Ali Khan, Sayeed Ahmad,* and Anuja Krishnan*

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ABSTRACT: Dengue is a tropical disease caused by the Dengue virus (DENV), a positive-sense, single stranded RNA virus of the family Flaviviridae, which is transmitted by Aedes mosquitoes. The occurrence of dengue has grown dramatically around the globe in recent decades, and it is rapidly becoming a global burden. Furthermore, all four DENV serotypes cocirculate and create a problematic hyperendemic situation. Characteristic symptoms range from being asymptomatic, dengue fever to life-threatening complications such as hemorrhagic fever and shock. Apart from the inherent virulence of the virus strain, a dysregulated host immune response makes the condition worse. Currently, there is no highly recommended vaccine or therapeutic agent against dengue. With the advent of virus strains resistant to antiviral agents, there is a constant need for new therapies to be developed. Since time immemorial, human civilization has utilized plants in traditional medicine to treat various diseases, including infectious viral diseases. With the advancement in molecular biology, cell biology techniques, and bioinformatics, recent studies have tried to provide scientific evidence and determine the mechanism of anti-dengue activity of various plant extracts and plant-derived agents. The current Review consolidates the studies on the last 20 years of in vitro and in vivo experiments on the ethnomedicinal plants used against the dengue virus. Several active phytoconstituents like quercetin, castanospermine, α-mangostin, schisandrin-A, hirsutin have been found to be promising to inhibition of all the four DENV serotypes. However, novel therapeutics need to be reassessed in relevant cells using high-throughput techniques. Further, in vivo dose optimization for the immunomodulatory and antiviral activity should be examined on a vast sample size. Such a Review should help take the knowledge forward, validate it, and use medicinal plants in different combinations targeting multiple stages of virus infection for more effective multipronged therapy against dengue infection.

1. INTRODUCTION

Dengue is a widespread viral infection caused by the four dengue virus serotypes, DENV1, DENV2, DENV3, and DENV4. DENV is a member of the genus Flavivirus, family Flaviviridae, and is transmitted to humans via biting of an infected female Aedes aegypti mosquito, the primary vector species.1 In the mosquito, the virus replicates in the midgut cells and then is released into the hemocoel and disseminates to other tissues via hemolymph, ultimately reaching the salivary glands and releasing virions in the next bite to the human host. The person gets symptoms of dengue fever 5 days after being bitten by an infected mosquito, and these symptoms might last a week or more.2 Before 1970, only nine countries had experienced severe dengue epidemics. At present, the disease is endemic in more than 100 countries. According to a recent estimate, around 390 million dengue infections could occur every year, of which 96 million manifest clinically. The Dengue virus infection poses a threat to 3.9 billion individuals in 128 nations.3 Almost 75% of the global population exposed to dengue live in Asia-Pacific.4 Early symptoms of a dengue-infected individual include fever, headache, rash, nausea, and joint and musculoskeletal pain.5 The primary manifestations of the disease include capillary leak syndrome or plasma leakage due to endothelial cell dysfunction, hemorrhagic tendencies, leukopenia, and thrombocytopenia, which is mainly seen in dengue hemorrhagic fever (DHF).6 The most severe Dengue shock syndrome (DSS) stage occurs during or shortly after symptom onset and is accompanied by transient hypotension or a weak pulse (less

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than 20 mmHg) with cold, clammy skin (in the early stage of shock), later being fatal in a few cases. The WHO guidelines for 2009 state that a rapid decline or platelet count below 150 000/mm$^3$ of blood is one of the indicators of clinical dengue worsening.

2. VIRUS STRUCTURE, FUNCTION, AND LIFE CYCLE

DENV is enveloped, positive-sense, single-stranded RNA virus which has four closely related serotypes (DENV-1 to -4) that are antigenically distinct. Each DENV serotype is subdivided into different genotypes and clades based on the divergence of viral gene sequences. Around 10.9 kb of the DENV genome encodes one open reading frame (ORF) with three structural (capsid, precursor membrane, and envelope) and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The structural protein envelope glycoprotein (E) is the main target for neutralizing antibodies and involves receptor binding and entry to the host cell by fusion.

The first step of virus infection involves attachment of DENV to the host cell surface and entry by receptor-mediated endocytosis. The DIII domain of E protein is the receptor-binding domain that binds to the host receptor, like DC-SIGN, mannose receptor, heparin sulfate, and many others. In the endosome, acidic pH results in activation and trimerization of the E protein in the virion, resulting in the fusion of the viral and cell membranes. The nucleocapsid containing the RNA genome is released into the cytoplasm, where it gets translated into a single polyprotein that is processed cotranslationally and post-translationally by viral NS3 protein and host proteases. Replication of the genome occurs on intracellular membranes. Assembly of immature virus particles occurs on the surface of the endoplasmic reticulum. Noninfectious, immature viral, and subviral particles are transported through the trans-Golgi network. The immature virion particles are then cleaved by the host protease furin, resulting in mature, infectious particles, which are subsequently released from the host cell by exocytosis. In the immature virus, the prM protein forms protruding trimers with E, which creates a "spiky" appearance, whereas in the mature virion, the membrane protein (M) sits below the E protein with a "smooth" surface.

Figure 1 illustrates the stages of the DENV life cycle and points where drugs can inhibit DENV infection.

3. HOST IMMUNE RESPONSE AND VIRUS PATHOGENESIS

Viral RNA is sensed by two host sensors, one belonging to cytoplasmic retinoic acid-inducible gene I (RIG-I) like receptors (RLR) and another belonging to endosome toll-like receptors (TLR-3 and TLR-7). RIG-I and melanoma differentiation-associated protein 5 (MDA5) recruits the adaptor mitochondrial antiviral-signaling protein (MAVS) pathway to activate within the cytoplasm on dengue infection. This MAVS activation triggers phosphorylated TANK-binding kinase-1 (TBK1) and IκB kinase-ε (IKKe) complex to phosphorylate interferon regulatory factors 3 and 7 (IRF3 and IRF7). The phosphorylated IRF3 and IRF7 enter the nucleus to trigger the production of type I interferons (IFNs)
such as IFN-β. The interferon (IFN) system is the primary host defensive mechanism by which the innate immune defense system gets activated against viruses. The IFN system consists of type I interferons (IFN-α and IFN-β), type II interferon (IFN-γ), and type III interferons (IFN-λ1−4), also known as IL-28A−C, IL-29. Type I IFNs (IFN-α and IFN-β) are the primary IFNs generated in almost all nucleated cells within hours of viral infections. Meanwhile, dsRNA formed during viral replication activates endosomal primary TLR-3, which thereby causes phosphorylation of TIR-domain-containing adapter inducing IFN-β, interacting with TNF-receptor-associated factor 3 (TRAF3) and TBK1/IKKe to induce IFNα/β-stimulating genes (ISGs) and chemokines. TLR7 recognition of ssRNA, including DENV genomic fragments, uses the myeloid differentiation primary response gene 88-dependent signal pathway (MyD88) by recruiting TRAF6 to activate inhibitor of nuclear factor-κB kinases (IKKa/IKKβ/IKKe) and trigger nuclear factor-xB (NF-xB) to ultimately produce IFNα, IFNβ, and proinflammatory cytokines (TNFα and IL6). Secreted type-I/III IFNs bind to their receptors IFNAR1/2, which further activates Janus kinase (Jak)—signal transducer and activator of transcription (STAT)-mediated signaling pathway leading to transcription of ISGs, which inhibit virus infection. The stimulation of IFN genes (STING), which function downstream of MAVS and upstream of TBK1, plays a vital role in activating IRF3 and nuclear factor-kappa B (NF-xB) (Figure 2).

To counteract the host antiviral actions, DENV has evolved strategies targeting various immune defense system steps, from sensing the foreign DNA/RNA to the induction, signaling, and manipulation of the IFN system. Dengue disease manifestation ranges from mild to severe cases. Several studies indicate that excessive inflammation contributes to the pathogenesis of severe dengue disease. DHF/DSS is thought to manifest in the context of amplified and unbalanced production of cytokines termed as cytokine storm. Dysregulated cytokines ultimately target the vascular endothelium and eventually lead to a transient increase in vascular permeability, the occurrence of hemorrhagic manifestations, and hemoconcentration, a hallmark of DSS. Many inflammatory cytokines and chemokines, especially IL-6, IL-8, IL-1β, IP-10, CCL8, CXCL9, CXCL16, and MCP-1, and immunosuppressive cytokines, especially IL-10, are elevated, resulting in increased vascular permeability in severe dengue cases (Figure 3). These complex interaction networks of several cytokines regulate the pathogenesis of dengue, and their fine modulation determines the disease outcome. The immunomodulation of the virus-induced hyperinflammation-caused severe cases of dengue are being explored for therapeutic intervention.

Antiviral candidates are tested in vitro using various biochemical and cell-based assays, indicating their potential as therapeutic. A few of the most used assays are discussed below and with illustration in Figure 4.

4. ANTI-VIRAL ASSAYS

4.1. Cell-Based Assays.

4.1.1. Cytopathic Effect. The cytopathic effect (CPE) refers to cellular changes induced by a virus that can be microscopically visible. The infected monolayer cells gradually deteriorate
with the changes like swelling, shrinkage of cells, syncytia (cell–cell fusion), and inclusion bodies. The cytopathic effect of any agent is measured as the median tissue culture infectious dose (TCID 50). TCID 50 is defined as diluting a virus that can infect 50% of the given cell culture.

4.1.2. Plaque Assay. This is a classical virology technique used to quantify infectious viral particles and is based on the fact that viruses can induce cell lysis. In a plaque assay, serial dilution of the virus is inoculated to susceptible cells for a few hours. Cells are then covered with a nutrient medium containing agar or methylcellulose, restricting viruses released from infecting neighboring cells. Each infectious particle generates a plaque, a circular zone of infected cells. The plaque eventually becomes large enough to be seen with the naked eye. Dyeing agents for living cells are frequently utilized to improve the contrast between the living cells and the plaques, which will be transparent. Plaque assays are reported as plaque-forming units (PFU) per measure volume. The conventional plaque assay is time-consuming and labor-intensive, with low throughput. Therefore, a faster version of these assays has been developed, such as the focus forming assay.22

4.1.3. Focus Forming Assay. The focus forming assay relies on detecting viral proteins by immune staining techniques, offering the unique advantage of detecting viruses but not producing cell damage. The procedure is similar to plaque assay until overlaying stage. Focus assay utilizes fluorescently labeled antibody-based staining and may detect infected but not necessarily dead cells. For more quantitative analysis, flow cytometry is used to measure the ability of a virus to infect cells. Based on the same principle, microneutralization assays based on an ELISA, flow cytometry, and ELISPOT enhance sensitivity by using antibodies against DENV proteins inside host cells.23–25

4.1.4. Viral Replication. The inhibitor effect is also assessed by quantifying viral replication. Viral RNA is extracted from cells or supernatants using a viral RNA extraction kit. cDNA is synthesized using the reverse transcriptase (RT) enzyme. The cDNA is amplified using DENV-specific primers, and a fluorescent dye that intercalates between the DNA bases and binds within double-stranded DNA molecules. Real-time PCR is a quantitative PCR method because the magnitude of fluorescence can be detected at the end of each amplification cycle. A real-time-PCR read-out is given as Ct (cycle threshold) value, the number of PCR cycles for the fluorescent signal to cross the threshold value. Ct values are inversely proportional to the amount of nucleic acid present.26,27

In order to understand which step of viral lifecycle is blocked by the inhibitor, time of drug addition (TDA) experiments are performed. At different time points during virus infection, an antiviral agent is administered to the virus or host cells.28 (1) Before viral infection; cells are pretreated with an antiviral agent to see whether the drug can block the viral receptor and inhibit viral attachment to the host cells. (2) Pretreatment of the virus with an antiviral substance, followed by inoculation of the treated virus to the cells, assesses the virucidal activity of the antiviral substance. The virucidal assay is performed to determine whether a test compound inactivates virus outside

![Figure 3. Immune response observed in mild and severe dengue disease.](image)

![Figure 4. Assays used for assessing antiviral activity.](image)

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of cells or if the compound inactivates the virus before it infects the cells. (3) Cells are cotreated with virus and antiviral drug to investigate the antiviral effect on the virus entrance processes, including virucidal (neutralizing) activity and inhibition of viral attachment and penetration to the cells. (4) Treatment of antiviral agent after virus infection examines the antiviral effect during the postentry steps, such as translation and replication, virion assembly, and release from the cells.29

4.2. Biochemical Assays. NS3 and NS5 are DENV enzymes that have critical role in the DENV life cycle. Candidates with anti-NS5 or NS3 activity can be a potential therapeutic agent. These biochemical enzymatic assays are easy to perform in a test tube without requiring a cell culture facility or live virus and produce precise interpretable results.30

4.2.1. NS3 Activity. NS3 together with cofactor, NS2B, constitute a serine protease that cleaves viral polyprotein to individual proteins. The NS2B/NS3 protease complex is essential for viral replication and is a primary target for developing antiviral drugs. NS3 protein can be expressed in E. coli or baculovirus system and purified. NS3 activity is measured using a synthetic fluorogenic/chromogenic substrate containing amino acid sequence derived from the NS2B/NS3 site. The rate and amount of cleavage are evaluated spectrophotometrically. IC_{50} values are determined for inhibitors from substrate titration experiments performed in the presence of increasing inhibitor concentration using Dixon plots.31,32

4.2.2. NS5 Polymerase Activity. NS5 is a RNA-dependent RNA polymerase (RdRp) which replicates viral RNA. Recombinant NS5 RNA polymerase activity is evaluated by the elongation assay. The most frequent method used is the scintillation proximity assay (SPA). In this assay, a biotin-labeled primer is annealed to a poly rC template, and the primer extension is initiated in the presence of 3H-GTP and NS5 polymerase. The newly synthesized RNA incorporating radioactive GTP is captured through biotin binding to streptavidin-coupled SPA beads, and a liquid scintillation counter detects the captured radioactivity. Other variations give fluorescence-based read-out instead of radioactivity.33 De novo RNA synthesis of NS5 is measured using a DENV-2 sub genomic RNA template.

Apart from evaluating the direct effect of the inhibitor on virus infection, there is a search for inhibitors that could modulate DENV-induced hyperimmune response, thus alleviating the “cytokine storm” seen in severe dengue cases. In order to test whether an inhibitor has an immunomodulatory effect, cytokine levels of the host in the presence of an inhibitor on dengue infection are measured by cytokine assay. The levels of cytokines in virus-infected supernatant or patient serum are quantified by cytokine sandwich ELISA which detects and quantifies the concentration of soluble cytokine and chemokine proteins. Cytokine gene transcription levels inside cells can be measured by RT-PCR using cytokine-specific primers.34

5. IN VIVO STUDIES

Since there is no appropriate animal model to imitate dengue sickness, particularly the severe forms (DHF/DSS), it is a major technical impediment to understanding the etiology and testing of any prospective candidate for DENV infection. Nonhuman primates show viremia on dengue infection but do not show any clinical signs. Also, the use of primate species has limitations, like the high cost and challenges of managing them. There is no appropriate mouse model for dengue since rodents do not exhibit dengue virus replication efficiently. The ICR suckling mouse was the initially used model obtained by serial passaging of DENV in the brain (intracranial), resulting in DENV-induced encephalitis and paralysis.35 It is still used; however, the biological relevance is questionable since DENV primarily infects the brain, whereas in reality, DENV brain infection is rare. Dengue infection in immunocompetent mice like Balb/C, A/J, and C57BL/6 is rare. However, inoculation with high viral load (VL) in C57BL/6 detects virus in serum, liver, and the brain, though the viral load is low. Artificial viremia induced in mice by inoculation with K562-infected cells has been reported and used.36 Humanized mice animal models transplanted with human cells targeted explicitly by the virus during natural infection have developed clinical signs of DF like fever, erythema, and thrombocytopenia.37 DENV subverts interferon signaling in humans, which is not achieved in mice, and therefore, it is unable to replicate. AG129 mice are deficient in IFN alpha/beta and gamma signaling, so when infected with DENV, clinical isolates show VL in serum, liver post 3 days infection (DOI), and death on day 7 with paralysis because there is a high viral load in the CNS.38

6. MEDICINAL PLANTS IN DENGUE THERAPEUTIC RESEARCH

Medicinal plants have been widely used throughout the world, especially in developing countries, to treat various infectious and noninfectious ailments. Plant-based natural medicinal product usage is becoming popular even in developed countries. As per World Health Organization (WHO) estimates, 80% of the world’s population fulfills their healthcare needs from phytomedicinal sources.39 This is because plants and their derived products are often seen as natural, readily available, and having few side effects. Natural products obtained from plants have long been one of the most important sources of “lead” compounds for the pharmaceutical industry, with up to 40% of modern medicines obtained from natural sources employing either the natural substance or a synthetic version. Medicinal plants include a variety of chemical compounds with a variety of biological capabilities, including the potential to inhibit the replication cycle of various types of viruses. Until now, in the treatment of dengue, no licensed drug is available that targets the virus. The emergence of viral resistance to antiviral medicines necessitates discovering new efficient antiviral drugs. Various plants are reported in various traditional medicine systems to combat the dengue disease.

Traditional plants like Carica papaya and Euphorbia hirta have been used in different countries based on interpretation of the results they have found for generations.40 However, proof of concept and knowledge of the mode of action is lacking. Today for any agent to be used as any useful therapeutic product, it should be adequately quantified for its toxicity and efficacy and validated using modern lab methods to establish the dosage, the toxicity, and the mode of action. Also, the validation should be done in vivo and ex vivo in animal models mimicking the disease. In the last two decades, various studies using plants and their derived compounds have been conducted for their antiviral activity, including the dengue virus. Most of the research has been done in cell lines, with only a few in vivo validations. In vitro cell culture models are the primary screening point for the search for any inhibitor. The following sections describe extracts or/and isolated compounds from various families of medicinal plants that have been reported with antiviral activity. Table 1
| n. | plant name/part used/family | extract used/active constituents | assay method/mechanism of action | targeted DENV serotype and cell line used | study parameters | references |
|---|-----------------------------|---------------------------------|----------------------------------|------------------------------------------|-----------------|------------|
| 1 | Andrographis paniculata, aerial part, Acanthaceae | methanolic extract | assay: CPE | DENV-1, Vero cells | MNTD = 0.050 | 43 |
| 2 | Andrographis paniculata | andrographolides | assay: flow cytometry, RT- PCR, TDA MOA: inhibition at the postinfection stage, virus budding and secretion | DENV-1, DENV-2, HepG2 and HeLa cells | EC\textsubscript{50} = 21.3 μM | 70 |
| 3 | Acacia cateu, herb powder, Leguminosae | peptide extract | assay: FFU assay, TDA assay MOA: binding of the peptide to the virus inhibition at the early step of infection. | DENV-2, Vero and Huh7 cells | IC\textsubscript{50} = 0.18 μg/mL | 57 |
| 4 | Acorus calamus, leaves, Acoraceae | methanolic extract | assay: FFU | DENV-2, Huh7r1-1 cells | CC\textsubscript{50} = 42.493 μg/mL | 48 |
| 5 | Anacolosa pervilleana, leaves, Asclepiadaceae | methanolic extract | assay: NS5 polymerase assay MOA: inhibited DENV NS5 polymerase activity | DENV-1, DENV-2 | IC\textsubscript{50} = 3 μM | 82 |
| 6 | Arrabidaea pulchra, leaves, Bignoniaceae | ethanolic extract | assay: MTT | DENV-2, Vero and LLCMK-2 cells | EC\textsubscript{50} = 46.8 ± 1.6 μg/mL | 52 |
| 7 | Azadirachta indica, leaves, Meliaceae | azadirachtin aqueous extract | assay: CPE and viral RNA RT- PCR | DENV-2, C6/36 cells | MNTD = 1.897 mg/mL | 51 |
| 8 | Alternanthera philoxeroides, Amarantaceae | petroleum ether | assay: MTT assay and CPE | DENV-2, C6/36 cells | TD\textsubscript{50} = 47.43 | 42 |
| 9 | Allium sativum, Amaryllidaceae | diallyl disulfide (DADS) | assay: viral RNA RT-PCR, cytokine ELISA MOA: immunomodulatory activity | DENV-2, Huh-7 cells | reduced inflammatory cytokines (TNF-α, IL-8, IL-10) in DENV-2 infection | 75 |
| 10 | Basilia piloselloides, Lamiales | - | assay: plaque assay | DENV-1, DENV-2 Vero cells | IC\textsubscript{50} = 96 ± 17 μM | 83 |
| 11 | Boesenbergia rotunda, Rhamnaceae | methanolic extract | assay: NS2b/NS3 protease assay MOA: inhibited NS3 protease activity | DENV-1 | K\textsubscript{i} = 21 and 25 μM for 4-hydroxyphenyl duratin A and panduratin A, respectively | 84 |
| 12 | Castanospermum austral, seeds, Myrtaceae | castanospermine | assay: plaque, flow cytometry, subgenomic replicon assay MOA: inhibit secretion of virus particles | DENV-1, DENV-2, DENV-3, DENV-4, BHK-21 and Huh-7 cells | IC\textsubscript{50} = 85.7 μM (Huh-7 cells), IC\textsubscript{50} = 1 μM (BHK-21 cells) | 73 |
| 13 | Cassia fistula, aerial part, Leguminosae | methanolic extract | assay: plaque assay, NS1 ELISA, cytokine ELISA, MOA: Virucidal effect | DENV-1, DENV-2, DENV-3, DENV-4, Vero, LLCMK-2, and C6/36 cells | IC\textsubscript{50} ≤ 25 μg/mL | 76 |
| 14 | Clausothamnium kamtschatcense | Fucoidan | assay: FFU assay, dye-labeled virus binding assay MOA: inhibition of virus entry | DENV-2, DENV-3, DENV-4, BHK-21 cells Vero cells | IC\textsubscript{50} = 4.7, 500, and 365 μg/mL for DENV-2, 3, and 4, respectively | 58 |
| 15 | Coptis chinensis | - | assay: MTT assay | DENV-2, Vero cells | EC\textsubscript{50} = 26.4 μM | 64 |
| 16 | Cryptocarya aborea, bark, chartaceous A-F | chartaceous A-F | assay: plaque assay and TDA MOA: blocking the activity of NS3 protease, viral RNA replication/RNA dependent RNA polymerase, Vero cells | DENV-2 | IC\textsubscript{50} = 1.8 ±4.2 μM | 85 |
| 17 | Cryptocarya criptocarpa, sea-weed | ethanolic extract | assay: plaque assay and TDA MOA: inhibit NS5 polymerase activity | DENV-2, DENV-3, DENV-4, Vero cells | IC\textsubscript{50} = 1 μg/mL (DENV-2), IC\textsubscript{50} = 3.9 ±14.2 μg/mL (DENV-3) and IC\textsubscript{50} = 29.3–60 μg/mL (DENV-4) | 67 |
| 18 | Cymbopogon citratus, root | methanolic extract | assay: FFU assay | DENV-2, Huh7r1-1 cells | CC\textsubscript{50} = 183.74 μg/mL | 48 |
| 19 | Distellocarpea, leaves, fruit | ethanolic extract | assay: MTT assay, antioxidant activity EC\textsubscript{50} = 9.8 μg/mL (leaf extract) | DENV-2, Vero and LLCMK2 cells | EC\textsubscript{50} = 11.1 ± 1.6 μg/mL (fruit extract) | 52 |
| 20 | Flacourtia ramonchii, stem bark | betulinic acid 3β-caffeate | viral RNA replication/RNA dependent RNA polymerase (RdRp) polymerase assays | DENV RNA polymerase, Vero cells | IC\textsubscript{50} = 0.85 ± 0.1 μM | 82 |
| no. | plant name/part used/family | extract used/active constituents | assay method/mechanism of action | targeted DENV serotype and cell line used | study parameters | references |
|-----|-----------------------------|---------------------------------|----------------------------------|------------------------------------------|-----------------|------------|
| 21  | Ficus septica, fruit hartwood stem, and leaves | methanolic extract | assay: immunofluorescence | DENV-1 and DENV-2, A5-49, HepG2, HuH7.1 cells | IC_{50} = 3.05 ± 0.10 μg/mL | 44 |
| 22  | Gastrodia elata, WSS45-sulfated derivative of α-β-glucan | - | assay: MTT, RT-PCR, plaque assay, TDA MOA: inhibits early stage of virus life cycle | DENV-2, BHK-cells | EC_{50} = 0.68 ± 0.17 μg/mL | 64 |
| 23  | Gymnocalycium torulosus, red seaweed | degalactan hybrid | assay: plaque assay and TDA MOA: virucidal effect, inhibits early stage of virus infection - binding of the virus to receptor | DENV-1, DENV-2, DENV-3, DENV-4, HepG2, HuH-7, A549 cells | IC_{50} = 20 μM; also significant reduction of cytokine (IL-6 and TNF-α) and chemokine (RANTES, MIP-1β, and IP-10) | 58 |
| 24  | Garcinia mangostana, pericarp of fruit | ethanolic extract, α-mangostin | assay: immunofluorescence, flow cytometry, cytokine transcription MOA: immunomodulation | DENV-2 NS2B-NS3 protease, Vero cells | IC_{50} ≤ 100 μg/mL | 56 |
| 25  | Hedychium auricularia, Hemigrapta, leaves and stems | ethanolic and methanolic extracts | assay: plaque assay, RT-PCR, NS3 protease activity MOA: inhibited NS3 protease activity | DENV-2 NS2B-NS3 protease, Vero cells | IC_{50} = 0.19–1.7 μg/mL | 59 |
| 26  | Hippeastrum rhizomoides, leaves | ethanolic extract | assay: plaque assay, cytokine ELISA MOA: inhibited NS5 polymerase activity | DENV-2, human macrophages | IC_{50} = 50 μg/mL; also significant decrease in DENV infection and the release of cytokines | 86 |
| 27  | Houttuynia cordata, whole plants | aqueous extract | assay: viral RNA RT PCR, plaque assay, TDA assay MOA: decrease viral RNA production, virucidal and early stages of infection | DENV-2, HepG2 and LLC-MK2 | EC_{50} = 0.8 μg/mL | 62 |
| 28  | Leucaena leucocephala, seeds | aqueous extract | assay: MTT assay and immunofluorescence | DENV-1, C6/36 cells | at 37 mg/L, a 100-fold decrease in virus titer | 45 |
| 29  | Laurencia longiflora, leaves, stems, and roots | ethanolic and methanolic extracts | assay: plaque assay, RT-PCR, NS3 protease activity MOA: inhibited NS3 protease activity | DENV-2, Vero cells | IC_{50} ≤ 100 μg/mL | 56 |
| 30  | Mimosa scabrella, seeds | sulfated galactomannans | assay: MTT, assay and immunofluorescence | DENV-2, C6/36 cells | at 347 mg/L, a 100-fold decrease in virus titer | 45 |
| 31  | Momordica charantia, roots and entire fruits | methanolic extract | assay: MTT and CPE | DENV-1, Vero and B6 cells | MNTD = 0.20 mg/mL | 43 |
| 32  | Morus cymbosula, leaves and barks | ethyl acetate extract | viral RNA replication, assay: NS5 polymerase activity MOA: inhibited RNA dependent RNA polymerase (DENV-NS5 RdRp) activity | DENV-2 | at 1 μg/mL, inhibition of NS5 RdRp activity by 87% | 87 |
| 33  | Myrtus communis, seeds | methanolic extract | assay: real-time PCR, TDA MOA: effective at both pre and post-treatment | DENV-2, Vero cells | EC_{50} = 9.6 μg/mL (coumarin A) EC_{50} = 2.6 μg/mL (coumarin B) | 88 |
| 34  | Nephelium lappaceum | methanol extracts | assay: plaque assay, TDA MOA: virucidal effect | DENV-2, Vero cells | EC_{50} = 37.5 μg/mL and 10.1 μg/mL, for lupeol acetate and voacangine, respectively | 88 |
| 35  | Tabernaemontana cymosa, seeds | ethanolic extract lupeol acetate and voacangine | assay: FFU assay MOA: effective at post-treatment | DENV-2, HuH7-1 cells | EC_{50} = 25.33 μg/mL | 48 |
| 36  | Myristica fatua | methanolic extract | assay: plaque assay, plaque assay, time-of-addition assay, virucidal assay MOA: inhibits binding of the virus to the receptor at early stages of infection ELISA competitive binding assay confirmed geranium interaction with E-DIII with high affinity | DENV-2, Vero cells | IC_{50} = 1.75 μM | 68 |
| 37  | Nephelium lappaceum | geranium | assay: immunofluorescence, MOA: virucidal effect | DENV-1, HepG2 cells | MNTD = 23.44 μg/mL there was 75% inhibition | 53 |
| 38  | Oligum sanctum | methanol extracts | assay: CPE, plaque assay | DENV-1, DENV-2, DENV-3, DENV-4, Vero, LLCM, and C6/36 cells | IC_{50} ≤ 25 μg/mL | 76 |
| 39  | Phyllanthus amarus, plant | methanolic extract | assay: plaque assay, NS1 ELISA, cytokine ELISA MOA: Virucidal effect | DENV-2, Vero cells | CC_{50} = 1000.0; | 89 |
| 40  | Psidium guajava | ethanolic extracts catechin | assay: FFU assay, TDA MOA: catechin inhibited both at early and late stage of infection | DENV-2, Vero cells | EC_{50} = 7.8 |
| s. no. | plant name/part used/family | extract used/active constituents | assay method/mechanism of action | targeted DENV serotype and cell line used | study parameters | references |
|-------|-----------------------------|---------------------------------|----------------------------------|------------------------------------------|-----------------|-----------|
| 41    | Polygonum cuspidatum, rhizomes | methanolic extract | flow cytometry-based viral infection assay, cell-cell spread assay, TDA MOA: virucidal activity, Block the viral attachment and entry/fusion | DENV-2, Vero, and Huh-7 cells | CC<sub>50</sub> = 227.7 ± 1.1 μg/mL, EC<sub>50</sub> = 8.1 ± 1.0 μg/mL, SI = 28.1 | 66 |
| 42    | Persea americana, fruit (2,4 R)-1,2,4-trihydroxyheptadec-16-yn-1 (THHY) extracted from fruit | | DENV RT PCR, Western blot of cytokine protein, ISER-driven luciferase reporter assay MOA: immunomodulation | DENV-1, DENV-2 | EC<sub>50</sub> = 10.98 ± 1.9 μM | 81 |
| 43    | Quercus laitiana, gall | crude methanol extracts/methyl gallate | MTT, CPE and NS2B/NS3 protease assay DENV-2, NS3 protease, C6/36 cells MOA: inhibited NS3 protease activity | DENV-2 NS2B-NS3 protease, Vero cells | IC<sub>50</sub> = 30.1 μg/mL | 47 |
| 44    | Senna angustifolia, plant leaves and stems | ethanolic extract | plaque assay, RT-PCR, NS3 protease activity MOA: inhibited NS3 protease activity | DENV-2 NS2B-NS3 protease, Vero cells | IC<sub>50</sub> = 8.1 ± 1.0 μg/mL | 81 |
| 45    | Schisandra chinensis | schisandrin-A | viral RNA RT-PCR, plaque assay, cytokine RT-PCR MOA: immunomodulation increased STAT1/2 phosphorylation, thereby increasing IFN-α gene expression | DENV-2, DENV-3, DENV-4, Vero cells | IC<sub>50</sub> = 28.1 ± 0.42 μM | 77 |
| 46    | Syzygium samarangense, dried leaves | aqueous extract 5-hydroxy-7-methoxy-6-methylflavanone (FNSY) | plaque, TDA, fusion inhibition assay MOA: inhibition early stage of infection most likely at fusion stage | DENV-2 DENV-4, Vero cells, and LLCMK-2 cells | EC<sub>50</sub> = 15.99 ± 5.38 μM | 69 |
| 47    | Trigonostemon moncherric, bark and wood | trigocherrin A1, A, and B | not mentioned | IC<sub>50</sub> = 1.27, 3.1, and 16 μM for trigocherrin A1, A, and B, respectively | 90 |
| 48    | Tridax procumbens, stem | ethanolic extract | plaque assay, RT-PCR, NS3 protease activity MOA: inhibited NS3 protease activity | DENV-2 | IC<sub>50</sub> = 25.6 ± 3.8 μg/mL for NS3 protease activity | 56 |
| 49    | Tripterygium wilfordii, root extracts, celastrol | root extracts, celastrol | Viral RNA RT-PCR, host gene RT PCR, phosphoprotein Western blotting, plaque assay, cytokine ELISA MOA: immunomodulation, celastrol induces anti-viral IFN-α gene expression and protein secretion | DENV-1, DENV-2, DENV-3 DENV-4, Huh-7 cells | IC<sub>50</sub> = 0.08-0.19 μM | 91 |
| 50    | Uncaria tomentosa, stem barks | alkaloid fraction, hydroethanolic extract | flow cytometry, cytokine ELISA MOA: immunomodulation, reduced TNF-α, IL-10, IFN-α production levels | DENV-2 human peripheral blood monocytes | at 1 μg/mL, significant reduction in TNF and IFN production and a reduction in DENV infection | 79 |
| 51    | Uncaria rhynchophylla | hirsutine | FFU, TDA MOA: inhibits the viral particle assembly, budding, or release step | DENV-1, DENV-2, DENV-3, DENV-4, BHK-21, and A549 cells | EC<sub>50</sub> = 10 μM | 72 |
| 52    | Vernonia tinina, leaves | methanolic extract | plaque assay, RT-PCR, NS3 protease activity MOA: inhibited NS3 protease activity | DENV-2 NS3 protease | IC<sub>50</sub> = 23.7 ± 4.1 μg/mL | 56 |
| 53    | Zostera marina | zosteric acid, CF-238 | plaque assay, TDA MOA: inhibition at an entry step in the viral life cycle | DENV-1, DENV-2, DENV-3 DENV-4, Vero cells | IC<sub>50</sub> = 3.3 Mm (ZF) | 65 |
| s. no. | plant species | extract/compound used | DENV serotype | study parameters | animal strain | references |
|-------|---------------|------------------------|---------------|-----------------|---------------|------------|
| 1     | *Azadirachta indica* | leaves’ extract | DENV-2 | at MNTD of 120–30 mg/mL, the leaf extract inhibited DENV replication as observed by the absence of viral RNA by RT-PCR and DENV related clinical symptoms of mice | suckling mice | 51 |
| 2     | *Carica papaya* | freeze-dried *Carica papaya* leaf | DENV-2 | DENV2 infected mice treated with 500 and 1000 mg/kg freeze-dried *Carica Papaya* leaf juice resulted in a decrease in inflammatory cytokines in the liver (CCL6, CCL8, CCL12, CCL17, IL1R1, IL1RN/IL1Ra, NAMPT and PF4/CXCL4) | AG129 mice | 92 |
| 3     | *Castanospermum australe* | castanospermine | DENV-1, DENV-2, DENV-3, DENV-4 | A/J mice were infected with mouse-adapted DENV-2 via the intracranial route daily for 10 days. Mice were treated with a range of doses of 0.2, 1, 5 mg of castanospermine, gives survival rates of 25%, 90% and 85% in mice | A/J mice | 73 |
| 4     | *Cissampelos pareira* | methanolic extract of aerial part | DENV-1, DENV-2, DENV-3, DENV-4 | mice infected with brain-adapted DENV were administered intraperitoneally with methanol free Cipa extract twice a day for 5 days; compared with the placebo-treated group, the level of protection (median survival time) by the 250 mg/kg dose was statistically significant (p = 0.01) | AGI29 mice | 76 |
| 5     | *Lonicera japonica* | honeysuckle aqueous extract | DENV-2 | treatment of aqueous honeysuckle extract before or after intracranial injection with DENV2 showed decreased NS1 RNA and protein expression levels accompanied by alleviated disease symptoms, decreased virus load, and prolonged survival time | ICR suckling mice | 93 |
| 6     | *Schisandra chinensis* | schisandrin A | DENV-2 | schisandrin A decreases the mortality of DENV-infected ICR suckling mice, and the survival rate of DENV-infected mice treated with schisandrin A reached 80% | ICR suckling mice | 77 |
| 7     | *Tripterygium wilfordii* | celastrol | DENV-2 | celastrol at a concentration of 0.1 mg/kg protected 80% of the mice against infection-induced lethality and related illness; celastrol induced an antiviral interferon response with significant increase in IFN-α-2, IFN-α-5, gene expression levels | ICR suckling mice | 91 |
| 8     | *Persea americana* | 1,2,4-trihydroxyheptadec-16-yn (THHY) | DENV-2 | TTHY (5 mg/kg) significantly decreased clinical scores (about 40%) and increased the survival rate (60%) of DENV infected mice as compared to control mice | ICR suckling mouse | 81 |
summarizes the studies conducted in cell lines and a biochemical assay system. Table 2 presents studies of medicinal plants conducted on in vivo systems, and Figure 5 illustrates the active compound with antidengue activity discussed in this paper.

There are various reports with plant extracts and isolated compounds exhibiting inhibition of dengue infection. The following plants and their isolated compounds have been shown to have antidengue activity as assessed by CPE, plaque assay, or FFU assay, but the precise stage or mode of action was not investigated.

*Andrographis paniculata* (family *Acanthaceae*) is a native herb of Southern and Southeastern Asia and is traditionally used for the common cold, throat infection, osteoarthritis, and ulcerative colitis. A study by Tang et al. reported that a methanolic extract of the aerial part of *A. paniculata* at a maximum nontoxic dose (MNTD)- 0.050 mg/mL was able to show a 75% inhibitory effect against DENV-1 in Vero cells by the CPE assay.

**Figure 5.** Molecular structure of some phytoconstituents or active compounds with already known antidengue activity.
**Alternanthera philoxeroides**, a native species found in South America, are rich in coumarin. The coumarin extract [TD (50) = 535.91] and petroleum ether [ED (50) = 47.43] showed an inhibitory effect against DENV (serotype not mentioned) in the MTT assay.42

**Momordica charantia** (bitter melon) belongs to the family Cucurbitaceae and is predominantly found in Asia, Africa, and the Caribbean. Leaves and green fruits have been used to fight cancer, diabetes, and many infectious diseases. A methanolic extract of **Momordica charantia** showed an inhibitory effect against DENV-1 at MNTD of 0.20 mg/mL in Vero E6 cells.43

**Ficus sepica**, family Moraceae, is traditionally used as a folk medicine for headache, fever, rheumatoid, cold, cough, bacterial, and fungal diseases. Experiments conducted by Huang et al. demonstrated that methanol extracts of fruit, heartwood, leaves, and stem from **Ficus sepica** inhibited DENV-1 and DENV-2 infection, with an IC50 of 3.05 ± 10.75μM.44

Ono and group reported that two galactomannans extracted from seeds of **Mimosa scabrella** (BRS) and **Leucaena leucocephala** (LLS) exhibited antidiuretic activity against DENV-1 in C6/36 cells. BRS at a concentration of 347 mg/1 and LLS at 37 mg/L could reduce viral infection about 100-fold.45

**Quercus lusitanica** (family Quercaceae) is a small tree or shrub found in the Mediterranean area, whose galls have been shown to have medicinal properties such as astringent and antidiabetic antipyretic and anti-Parkinsonian activities.46 In C6/36 cells, crude methanol extracts of **Q. lusitanica** inhibited DENV-2 infection at a TCID50 of 1–1000. At a TCID50 of 1000, methyl gallate isolated from fractionated crude extracts of **Q. lusitanica** showed a 96% inhibition at the MNTD of 100 μg/mL.47

**Acorus calamus** (family Acoraceae) has been used in traditional medicine for centuries to treat digestive disorders and pain. *Cymbopogon citratus*, commonly known as lemongrass, is native to Maritime Southeast Asia. *Myristica fatua* (family Myristicaceae) seeds are used in traditional medicine to treat headaches and other sicknesses. A study by Rosmalena et al. revealed that the methanolic extracts of **A. calamus**, **C. citratus**, and **M. fatua** at a dose of 20 μg/mL completely inhibited DENV-2 infection. **C. citratus** and **M. fatua** had EC50 values of 29 and 25 μg/mL, respectively.48

**Arrabidae Pulchra** (Cham.) Sandwith, is a vegetable consumed by people in the Caribbean. The ethanolic extract of leaves showed 80% inhibition at 100 μg/mL toward DENV-2 in Vero and LLCMK-2 cells in the MTT assay.49 Bioguided fractionation of this extract revealed that arylpropanoid glycoside derivatives, verbascoside, caffeoyl callerianin, and a terpenoid, ursolic acid, are responsible for this antiviral activity.

**Azadirachta indica** or Neem (family Meliaceae) is mainly found in the Indian subcontinent. Traditionally it is used as an anti-inflammatory, antiarthritic, antipyretic, antifungal, and antibacterial agent.50 An aqueous neem leaves' extract at its MNTD of 1.897 mg/mL inhibited DENV-2 in C6/36 cells by 100–10,000 TCID50.51 A bioguided isolation of fruit ethanolic extract of **Distictella elongata** (Bignoniaceae) revealed that a mixture of pectolinarin and acacetin-7-O-rutinoside showed anti-DENV-2 activity with an EC50 11.1 ± 1.6 μg/mL.52

**Ocimum sanctum**, popularly known as “holy basil”, has long been used to treat and prevent ailments like cough, fever, and ulcers. The methanolic extract exhibited inhibitory activity against DENV-1 with a MNTD of 23.44 μg/mL and showed around an 8-fold reduction in plaque assay.53 Stachyonic acid derived from *Basilicum polystachyon* showed antidiuretic activity with an IC50 = 1.4 μM in Vero cells.54

**Pavetta tomentosa** belongs to the family Rubiaceae and is distributed in India. The bark of *P. tomentosa* is used to treat visceral blockages in children, either as a decoction or in pulverized form. Decoctions of leaves are used to relieve the aches of hemorrhoids. *P. tomentosa* acetone leaf extract showed inhibitory activity against DENV-1 in C6/36 cells at CC50 = 125 μg/mL using MTT assay.55

A study by Rothen et al. showed that a methanolic extract of **Vernonia cinerea** leaves, an ethanol extract of **Tridax Procumbers** stems, and to less extent an ethanolic extract of *Senna angustifolia* leaves at 50 μg/mL were able to reduce the CPE of the DENV-2-infected cells effectively. **Tridax Procumbers** and **Vernonia cinerea** extracts showed a considerable reduction in plaque formation of about 80.6% ± 6.1 and 64.0% ± 9.4, respectively.56

The secondary metabolites of medicinal plants comprise various compounds with a wide range of biological activities. The active compounds have been identified in a few studies and showed a wide range of activity against DENV. The isolated products belonged to various chemical classes such as sulfated polysaccharides, flavonoids, quercetin, alkaloids, and terpenes. The chemical structures of the compounds from plants mentioned here are shown in Figure 5. Recent studies with plant extracts and isolated compounds have explored the mechanism of action for its infection inhibitory activity. Employing a time-of-drug-addition assay, it is possible to understand the possible point of inhibition in a virus life cycle.

### 6.1. Plants and Their Phytoconstituents Inhibiting Virus Attachment or Entry to Cell

The virus’s attachment to the host cell membrane and its entry into the host cell is the earliest stage of any viral infection. Inhibitors acting at this stage can be most efficient in inhibiting virus infection.

**Acacia catechu** (Family: Mimosaceae) is primarily found in India and Southeast Asia. It is used as a therapeutic for anti-inflammatory and antidiarrheal purposes. A study by Panya et al. reported that a crude peptide extract at 1.25 μg/mL could reduce virus production by 100-fold. Two isolated bioactive peptides at 50 μM could inhibit DENV foci formation by more than 90% and were found to be inhibitory against all four DENV serotypes. A time-of-drug-addition assay further revealed that inhibition occurred at the early stages of virus infection. The most effective peptide (designated Pep-RTYM) inhibited DENV infection with a half-maximal inhibition concentration of 7.9 μM.57

**Cladosiphon okamuranus** belongs to the family Chordaraceae. Fucoidan derived from *Cladosiphon okamuranus* was found to inhibit DENV-2 infection in BHK-21 cells by FFU assay.58 A dye-labeled virus binding assay revealed that fucoidan inhibited the binding of the virus to the cell. Pujol and group revealed that the di-galactan hybrids extracted from the red seaweed, *Gymnogongrus torulosus*, exhibits anti-DENV-2 activity in Vero cells with an IC50 of 0.19–1.7 μg/mL using plaque assay.59 A time-of-drug-addition assay revealed that the compounds have virucidal activity and inhibit the binding of the viral protein to the receptor.

**Houttuynia cordata** is a vegetable consumed by people in East and Southeast Asia. Its therapeutic use includes chronic sinussitis and allergy.60,61 Aqueous extracts from *Houttuynia*
cordata at concentrations as low as 10–40 μg/mL exhibited a significant protective effect by directly blocking the virus (virucidal) and decreasing virus replication.62

A sulfated derivative of an α-t-glucan, WSS45, from the Gastrodia dryata significantly reduced DENV-2 infection in BHK cells with an EC_{50} value of 0.68 ± 0.17 μg/mL. A time-of-drug-addition assay revealed that drug inhibition occurred at the early stages of DENV infection.

Zosteric acid (ZA) and an active compound CF-238, isolated from Zostera marina (marine eelgrass), exhibit antiviral activity. ZA displayed an IC_{50} of 2.3 μM against DENV-2. CF 238 showed IC_{50} values of 24, 46, 14, and 47 μM against DENV-1, DENV-2, DENV-3, and DENV-4, respectively. A time-of-drug-addition assay with CF 238 showed inhibition at an early stage of the viral life cycle.

Polygonum cuspidatum (family Polygonaceae) is used to treat cough, hepatitis, jaundice, amenorrhea, leucorrhrea, arthralgia, hyperlipidemia, scald, bruises, and snake bites. The methanolic extract from Polygonum cuspidatum rizomes (PCME) at 30 μg/mL inhibited DENV-2 infection without causing significant cytotoxicity. PCME displayed the virucidal activity of free virus particles and blocked the viral attachment and early entry/fusion events.

Two sulfated polysaccharides, the kappa/iota/nu carrageenan G3d and the d-galactan hybrid C2S-3 from the red seaweeds, Gymnogongrus griffithsi and Cryptomonas cereolate, exhibited antiviral activity in a plaque assay. The IC_{50} values were 1.0 μg/mL, 14 μg/mL, and 29.3–50 μg/mL with DENV-2, DENV-3 and DENV-4, respectively. A time-of-drug-addition assay demonstrated that G3d and C2S-3 inhibited only at the early stage of infection.

Nephelium lappaceum of the family Sapindaceae is found in tropical countries, such as Malaysia, Indonesia, the Philippines, Thailand, and Southeast Asian regions. Geraniin is the primary compound and is an ellagitannin. Geraniin inhibited DENV-2 infection by 50% at concentrations as low as 10 μM, it was able to completely inhibit the release of virions.

Castanospermine is a natural alkaloid obtained from the tree of the Castanospermum australae (black bean or Moreton Bay chestnut). Treatment of cells with castanospermine inhibited the yield of infectious DENV-2 in Huh-7 cell line with an IC_{50} of 85.7 μM and an IC_{90} of 1.0 μM in BHK-21 cells. There was reduced infectivity as measured by plaque assay but no change in replication as measured by RT-PCR thus confirming that inhibitory action was due to the reduction of secretion of viral particles from the host cell.

6.3. Plants Inhibiting Virus Infection Due to the Immunomodulatory Effect. An uncontrolled and generalized inflammatory response called ‘cytokine storm’ is observed in severe dengue patients (Figure 3). Apart from directly decreasing dengue infection, controlling inflammatory responses through immunomodulation can be one way to prevent severe disease.

Hippophae rhamnoides (Family Elaeagnaceae) is present in Europe and Asia. The alcoholic extracts of Hippophae rhamnoides leaves at 50 μg/mL significantly inhibited the DENV-2 infectivity in blood-derived human macrophages as assessed by plaque assay. Further, cytokine ELISA showed that there was also reduction in the secretion of inflammatory cytokines, TNF-alpha and IL-10.

Allium sativum or garlic (Family Amaryllidaceae) is used in high blood pressure, hyperlipidemia, and artherosclerosis.

Organosulfur compounds isolated from garlic, diallyl disulfide (DADS), diallyl sulfide (DAS), and allin were able to significantly reduce inflammatory cytokines (TNF-α, IL-8, IL-10) on DENV-2 infection in Huh-7 and U937 cells. Lipid peroxidation and iNOS, an indicator of oxidative damage, was also reduced on compound treatment.

A methanolic extract of Cissampelos pariera Linn (Cipa extract) and Phyllanthus amarus inhibited infection of all four DENV serotypes. Phyllanthus amarus exhibited IC_{50} of 3–20 μg/mL, and Cissampelos pariera displayed an IC_{50} of 1.2–11 μg/mL against all DENV serotypes. Pretreatment of DENV with an increasing concentration of Cipa extract revealed that infection is due to its direct virucidal effect. Further, Cipa extract efficiently suppressed the secretion of TNF-α and IL-1β levels, thus acting as an immunomodulating agent.

Schisandra chinensis (Turcz.) Baill is a widely used herbal medicine. It is mainly used as a sedative, analgesic, and antipyretic agent. It is also used to treat hyperlipidemia, heart conditions, and neurodegenerative diseases. Experiments by Yu et al. revealed that Schisandrin A could inhibit the replication of all four serotypes of DENV. Schisandrin A was most effective against DENV-2 at an EC_{50} of 28.1 ± 0.42 μM.

Schisandrin A significantly enhanced IFN-α gene expression in DENV infected cells. Schisandrin A also increased STAT1 phosphorylation. Downregulation of STAT1/2 using specific shRNAs restored DENV replication. Schisandrin A could increase the promoter activity of pISRE-Luc reporter plasmid carrying IFN-stimulated response element (ISRE)-driven firefly luciferase in the presence of DENV infection, thus confirming that Schisandrin A induces the antiviral interferon-stimulated gene expression for inhibition of DENV replication.

Bioactive compound α-mangostin (α-MG) from the pericarp of the mangosteen fruit (Garcinia mangostana Linn) inhibited DENV production and cytokine expression of HepG2 cells. Treatment of DENV-infected cells with α-MG (20 μM) significantly reduced the infection rates of all four
DENV serotypes by 47–55%. Furthermore, α-MG could markedly reduce cytokine (IL-6 and TNF-α) and chemokine (RANTES, MIP-1β, and IP-10) transcription.78

*Uncaria tomentosa* (a woody vine) found in Amazon and Central American rainforests has been traditionally used to treat several diseases like gastritis, gastric ulcers, cancer, arthritis, asthma, and inflammatory disorders. Crude hydroethanolic extract and alkaid fraction inhibited DENV-2 infection in human monocytes. A multiplex biometric immunoassay was employed for cytokine measurement. The alkaloid fraction at 100 μg/mL showed immunomodulatory function inhibiting proinflammatory cytokines, TNF-α and IFN-α.79

Celastrol (quinone methide triterpene) from root extracts of *Tripterygium wilfordii* (family Celastraceae) inhibited replication of all 4 DENV serotypes in the range of 0.08–0.19 μM. Celastrol is well-known for its immunomodulatory properties that boost the expression of IFN-2, IFN-5, and IFN-17 genes. Celastrol elevated STAT1/2 phosphorylation as measured by Western blot assay. A Jak1-specific inhibitor effectively attenuated the anti-DENV effect of celastrol. Silencing of STAT1 by shRNA resulted in decreased DENV replication. Celastrol induced antiviral IFN response through the JAK/STAT pathway.76

**Persea americana** (family Lauraceae), also known as avocado, is mainly found in tropical and subtropical regions. The fruit, stem, and leaf of avocado are widely used in ethnomedicine.80 A study conducted by Wu and their group revealed that (2 R,4 R)-1,2,4-trihydroxyheptadec-16-yne (THHY), extracted from avocado (*Persea americana*) fruit, inhibited replication of all DENV serotypes. THHY significantly suppressed DENV-2 RNA and protein synthesis, with an EC50 = 10.98 ± 1.9 μM. THHY treatment also increased STAT1 and STAT2 phosphorylation levels in the presence of DENV-2 replication.81 Using a NF-κB promoter-based reporter assay it was revealed that THHY dose-dependently induced NF-κB promoter activity upon DENV infection. Further, THHY induced IFN-α RNA level and secreted IFN-α in DENV-2-infected cells.

### 6.4. Plants Inhibiting Virus at Both Entry and Exit of Host Cell

Some of the inhibitors act at multiple points of the virus lifecycle. An ethanolic extract from the bark of *Psidium guajava* exhibited antiviral activity with CC50 1.0 mg/mL and EC50 7.8 μg/mL.89 Isolated bioactive compounds from the bark are gallic acid, quercetin, and catechin (50 μg/mL), which inhibited the production of infectious DENV-2 viral particles. The compound catechin was an efficient inhibitor of both pre- and post-treatment stages.89 Coumarin A and B isolated from *Mammee americana* seeds could inhibit DENV-2 infection at EC50 values of 9.6 and 2.6 μg/mL, respectively. The two compounds, lupeol acetate and voacangine, derived from the *T. cymosa* seeds, also significantly inhibited infection by DENV-2 in Vero cells with EC50 values of 37.5 and 10.1 μg/mL, respectively. Time-of-drug-addition assays revealed that coumarin A and B were effective as inhibitors in both pre and post-treatment experimental strategies, whereas lupeol acetate only significantly inhibited the DENV infection during the post-treatment strategy.88

As discussed previously, NS3 and NS5 are viral enzyme proteins critical for dengue virus replication and infectivity. Various compounds from medicinal plants have been studied for their inhibitory NS5 and NS3 activity.

### 6.5. Plants with NS3 Protease Inhibitory Activity

Compounds derived from *Boesenbergia rotunda* were tested for inhibitory activities toward DENV-2 NS3 protease activity, using fluorogenic peptide substrate Boc-Gly-Arg-Arg-MCA. 4-Hydroxyxanthotriol A and panduratin A are cyclohexenyl chalcone derivatives that inhibited DENV-2 NS3 protease activity at KIC values of 21 and 25 μM amounts, respectively.84 Pinocembrin and cardamonin were also able to inhibit NS3 protease activity though to a lesser degree, with KIC values of 345 ± 70 and 377 ± 77 μM, respectively. When used in combination, better inhibitory activity was seen, suggesting a synergistic effect. A mechanistic evaluation revealed that pinostrobin and cardamonin inhibited the NS3 protease via a noncompetitive mechanism while panduratin A and 4-hydroxyxanthotriol A inhibition was via competitive inhibition.

Rothen et al. screened around 19 extracts of different medicinal plants for potential NS3 inhibitory activity. Different extracts of *Hedyotis auricularia*, *Hemigraphis reptans*, and *Laurentia longiflora* exhibited inhibitory activities against DENV-2 NS2B-NS3 protease with all displaying IC50 values <100 μg/mL. An ethanolic extract of *Senna angustifolia* leaves, a methanolic extract of *Vernonia cinerea* leaves, and ethanolic extracts of *Tridax procumbens* stems were found to have the highest inhibitory activity compared with other extracts with IC50 values of 30.1 ± 3.4, 23.7 ± 4.1 and 25.6 ± 3.8 μg/mL, respectively.85

### 6.6. Plants with NS5 Polymerase Inhibitory Activity

*Myrtopsis corymbosa* (Family Rutaceae) are plants found in New Caledonia. Ethyl acetate crude extracts of barks and leaves of *M. corymbosa* were found to have anti NS5 activity as assayed by monitoring the incorporation of radiolabeled guanosine into a homopolymeric cytosine RNA template.87 Leaf and bark extracts at 10 μg/mL inhibited 78 and 92% viral RNA polymerase activity, respectively.

*Anacolus pervaillanca Bail* belongs to the family *Olacaceae* and is found throughout the tropics. Traditionally, it is used to treat schistosomiasis against syphilis and general weakness. Active compounds were identified from leaf extracts showing anti NS5 polymerase activity. Acetylenic acids, the octadeca-9,11,13-triynoic acid, (13E)-octadec-13-en-9,11-diynoic acid, (13E)-octadec-13-en-11-ynoic acid exhibited significant inhibitory activity. Chartaceones C (13E)-1,2,4-triynoxoheptadec-16-yne, (13E)-octadec-13-en-9,11-diynoic acid, (13E)-octadec-13-en-11-ynoic acid exhibited significant inhibitory activity with IC50 values around 3 μM.82

Highly oxygenated daphane diterpenoid orthoesters (DDO) with chlorinated moiety, Trigocherrin A and trigocherrholides A and B, isolated from the bark and the wood of *Trigonostemoncherrieri*, showed significant NS5 inhibitory activity with IC50 values of 12.7, 3.1, and 16.0 μM, respectively in NS5 polymerase assay.80

An *in vitro* screening of bark of *Cryptocarya chartacea* (new Caledonian plant) was done to identify compounds showing significant dengue virus NS5 inhibiting activity. Mono- and dialkylated flavanones, chartaceous A–F, along with pinocembrin exhibited significant NS5 inhibitory activity. Chartaceones C–F exhibited the most significant NS5 inhibiting activity, with IC50 ranging from 1.8 to 4.2 μM.85

### 7. PLANTS WITH ANTIDENGUE ACTIVITY: IN VIVO SYSTEM

After being tested *in vitro*, only few plant products have been taken forward for validation in *in vivo* models. *Azadirachta indica* Juss (neem leaves) extract at its MNTD 120–30 mg/mL with 100 LD50 doses of DENV-2 inoculated intracerebrally in sucking mice, resulting in inhibition of the virus replication as exhibited by the absence of dengue-related clinical symptoms (characteristic Dengue specific weight loss, slow
A study conducted by Norahmad et al. revealed that freeze-dried Carica papaya leaf (FCPLJ) extract in AG129 mice has an immunomodulatory effect. The AG129 mice infected intraperitoneally with DENV-2 (2 × 10^6 PFU) were fed orally with FCPLJ (500 mg/kg of mouse body weight) for 3 consecutive days after 24 h of dengue virus inoculation. The sign of illness was scored on the basis of lethargy, ruffled fur, moribund or paralyzed with limited activity, and inability to reach food-water. Plasma cytokines quantitation by immune assay showed that the treatment increased the plasma CCL2/MCP-1 level during the period of viremia. RT-PCR gene expression analysis of liver showed downregulation of mice inflammatory cytokine genes- CCL6/MRP-1, CCL8/MCP-2, CCL12/MCP-5, CCL17/TARC, IL1R1, IL1RN/IL1Ra, NAMPT/PBEF1, and PF4/CXCL4 in FCPLJ treated infected mice compared with untreated. AG129 mice infected intracranially with 10^7 PFU of a mouse-adapted DENV-2 strain developed hind limb paralysis and succumbed to fatal central nervous system infection within 11 days of inoculation. Castanospermine isolated from seeds of Castanospermum australae was shown to have a protective effect in the mouse model. Castanospermine treatment prevented mortality in a mouse model of dengue virus infection, with doses of 10, 50, and 250 mg/kg (of mouse body weight) per day being highly effective at promoting survival (P < 0.0001). AG129 mice were challenged with 10^6 PFU of DENV-2, which would be lethal 25 days postchallenge. Illness preceding death involved lethargy, ruffled hair, and hindlimb paralysis. Two hours after infection, a Cipa treatment of 10 mL/kg/dose was commenced and continued twice daily for 5 days. The median survival time (MST) of challenged mice orally treated with Cipa extract (methanol-free) twice a day for 5 days postchallenge was increased in a dose-dependent manner. The level of protection provided by the 250 mg/kg dose was statistically significant (p = 0.021) when compared with the placebo-treated group.

Honeysuckle (Lonicera japonica Thunb) aqueous extract before or after intracranial injection with DENV-2 in ICR suckling mice decreased clinical score significantly and prolonged survival time. In the study performed by Lee and group, ICR suckling mice were pretreated with aqueous extract of honeysuckle on day 4 after birth (twice a day) followed by intracranial (i.c.) injection of DENV-2 (2.5 × 10^3 PFU) on day 7, and all mice continuously drank aqueous honeysuckle extract until day 13. A decrease in viral load (42%) in the brain tissue and prolonged survival time was observed in the infected mice compared with the DENV-2-infected mice without extract treatment. Also, pretreatment of honeysuckle suppressed DENV-2 NS1 RNA and protein expression by 20% and 68%, respectively, in the brain tissue. In the post-treatment experiment, sucking mice were intracranially injected with DENV-2 (2.5 × 10^3 PFU) on day 6, followed by honeysuckle treatment starting on day 7, twice a day for 4 days. Honeysuckle treatment after DENV-2 infection significantly decreased the clinical scores of the infected mice on days 4 and 6 compared to the DENV-2-infected mice without honeysuckle from day 8 to day 10. Honeysuckle treatment resulted in a 25% reduction in NS1 RNA expression, a 52% reduction in NS1 protein expression, and a 30% viral titer in the brain tissue of the infected mice. Schisandrin, a bioactive compound derived from the fruit of Schisandra chinensis, was found to reduce DENV-infected mice’s illness symptoms and mortality effectively. Schisandrin A treatment showed a lower clinical score than the control mice. The survival rate of DENV-infected mice treated with schisandrin A reached 80% at 6 dpi compared to control. Schisandrin A increased IFN-α-2 and IFN-α-5 RNA levels, thus suggesting that schisandrin A inhibits DENV replication by stimulating IFN-mediated antiviral responses in vivo.

Celasotrol isolated from roots of Tripterygium wilfordii showed inhibition of DENV infection in vivo. Six-day-old ICR suckling mice (intracerebrally infected with 2.5 × 10^5 pfu of DENV-2) were treated with or without celastrol. Severe illness leading to death within 4–6 days dpi was noted in the DENV group. Celastrol at a concentration of 0.1 mg/kg protected 80% of the mice against life-threatening DENV-2 infection compared with the noncelastrol-treated mice. As observed in the in vitro studies, celastrol also showed IFN-modulating activity in vivo.

(2 R,4 R)-1,2,4-trihydroxyheptadec-16-yn-3-ol (THHY) extracted from Avocado fruit when infected in a DENV-infected ICR suckling mouse model resulted in an increased survival rate. Six-day-old ICR suckling mice infected with DENV-2 by intracerebral injection were treated with 5 mg/kg of THHY or saline. THHY significantly decreased clinical scores (about 40%) and increased the survival rate (60%) of DENV infected mice as compared with control mice.

Molecules with anti-dengue activity belonged to all different classes of compounds, including phenolic derivatives (4-hydroxy panduratin, geraniol, amangoitin, methyl gallate), alkaloids (castanospermine, hirsutine, palmitine), flavonoids (chartecones, pectolinarinandacacetin-7-O-ro tinosides, catechin, 5-hydroxy-7-methoxy-6-methyl flavanone), terpenoids (betalinic acid 3β-caffeate, lupeol acetate, celastrol, andrographolide), and polysaccharides (fucoidan, carrageenan, galactomanns).

### CONCLUSION AND FUTURE DIRECTION

There has been increased interest in plant products for their use in various ailments. Literature survey indicates that many recent studies report scientific evidence on their role and mechanism of inhibiting dengue virus infection in vitro and in vivo. The majority of studies have reported antidengue activity by decreasing dengue infection. Insight into the mode of action of antiviral agents is now almost a prerequisite for clinical development. Further, knowledge of the mechanism of action can aid in using a combination of plants that could be tested for inhibiting virus infection at various points. Taking these extracts or compounds forward in combination is essential for checking a synergistic effect. Most Southeast Asian countries are hyperendemic, with all four DENV serotypes circulating. Only few studies report the effect of plants using all four serotypes, and it would be of value if future studies were validated with all four DENV serotypes. In vitro methods are very cumbersome in virology, and there is a need to develop high-throughput assay systems where multiple products can be checked simultaneously, and there will be uniformity in the read-out. Also, antiviral assays should be done in more cell lines of physiological relevance like human monocytes. Apart from checking the effect on virus infection and host immune response, there are models available that check infection in vitro the vascular permeability before going to the in vivo model. Since dengue complication is because of hemorrhagic manifestation,
such assays would indicate if any natural product can protect against vascular damage. Many in silico studies have been done with plant products on their potential binding and inhibition of dengue viral proteins. It is highly recommended that these compounds be validated in vitro and later in vivo.

**AUTHOR INFORMATION**

**Corresponding Authors**

Anuja Krishnan — Department of Molecular Medicine, School of Interdisciplinary Sciences & Technology, Jamia Hamdard, New Delhi 110062, India; orcid.org/0000-0001-6538-2044; Email: Anuja.krishnan@jamiahamdard.ac.in

Sayeed Ahmad — Bioactive Natural Product Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India; Email: sahmad_jh@jamiahamdard.ac.in

**Authors**

Mohammad Altamish — Department of Pharmacology School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India

Muzayyana Khan — Bioactive Natural Product Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India

Mirza Sarwar Baig — Department of Molecular Medicine, School of Interdisciplinary Sciences & Technology, Jamia Hamdard, New Delhi 110062, India

Bharti Pathak — Department of Molecular Medicine, School of Interdisciplinary Sciences & Technology, Jamia Hamdard, New Delhi 110062, India

Veena Rani — Department of Sciences, Indira Gandhi National Open University (IGNOU), New Delhi 110068, India

Jamal Akhtar — Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, New Delhi 110058, India

A. Ali Khan — Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, New Delhi 110058, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c00625

**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Nonyong, P.; Ekalaksananan, T.; Phanthanawiboon, S.; Aromseree, S.; Phadungsuphat, J.; Nakayama, E. E.; Shida, T.; Sawaswong, V.; Payungporn, S.; Thaewnongkiew, K.; Overgaard, H. J.; Bangs, M. J.; Alexander, N.; Pintong, C. Dengue Virus in Humans and Mosquitoes and Their Molecular Characteristics in Northeastern Thailand 2016–2018. PLoS One 2021, 16 (9), e0257460.

(2) Ma, E.; Zhi, Y.; Liu, Z.; Wei, T.; Wang, P.; Cheng, G. Interaction of Viruses with the Insect Intestine. Annu. Rev. Virol. 2021, 8 (1), 115–131.

(3) Bhattacharjee, S.; Gething, P. W.; Brady, O. J.; Messina, J. P.; Farlow, A. W.; Moyes, C. L.; Drake, J. M.; Brownstein, J. S.; Hoen, A. G.; Sankoh, O.; Myers, M. F.; George, D. B.; Jaensch, T.; Wint, G. R. W.; Simmons, C. P.; Scott, T. W.; Farrar, J. J.; Hay, S. I. The Global Distribution and Burden of Dengue. Nature 2013, 496 (7446), 504–507.

(4) Carrasco, L. R.; Lee, Y. S.; Cook, A. R.; Lee, V. J.; Thein, T. L.; Go, C. J.; Lye, D. C. Predictive Tools for Severe Dengue Conforming to World Health Organization 2009 Criteria. PLoS Negl. Trop. Dis. 2014, 8 (7), No. e2972.

(5) Kularatne, S. A. M. Dengue Fever. BMJ. 2015, h4661.

(6) Nelwan, E. J. Early Detection of Plasma Leakage in Dengue Hemorrhagic Fever. Acta Med. Indones 2018, 50 (3), 183–184.

(7) Hosseini, S.; Oliva-Ramírez, J.; Vázquez-Villegas, P.; Rodríguez-García, A.; Muñoz-Soto, R. B.; Aghamohammadi, N.; Martínez-Chapa, S. O. Dengue Fever: A Worldwide Threat An Overview of the Infection Process, Environmental Factors for a Global Outbreak, Diagnostic Platforms and Vaccine Developments. Curr. Top. Med. Chem. 2018, 18 (18), 1531–1549.

(8) Narvaez, P.; Gutierrez, G.; Pérez, M. A.; Elizondo, D.; Nuñez, A.; Balmaseda, A.; Harris, E. Evaluation of the Traditional and Revised WHO Classifications of Dengue Disease Severity. PLoS Negl. Trop. Dis. 2011, 5 (11), No. e3197.

(9) Bell, S. M.; Katzelnick, L.; Bedford, T. Dengue Genetic Divergence Generates Within-Serotype Antigenic Variation, but Serotypes Dominate Evolutionary Dynamics. Elife 2019, 8, e42496.

(10) Yenamandra, S. P.; Koo, C.; Chiang, S.; Lim, H. S. J.; Yeo, Z. Y.; Ng, L. C.; Hapuarachchi, H. C. Evolution, Heterogeneity and Global Dispersal of Cosmopolitan Genotype of Dengue Virus Type 2. Sci. Rep. 2021, 11 (1), 13496.

(11) Zhang, X.; Jia, R.; Shen, H.; Wang, M.; Yin, Z.; Cheng, A. Structures and Functions of the Envelope Glycoprotein in Flavivirus Infections. Viruses 2017, 9 (11), 338.

(12) Cruz-Oliveira, C.; Freire, J. M.; Conceição, T. M.; Higa, L. M.; Castanho, M. A. R. B.; Da Poian, A. T. Receptors and Routes of Dengue Virus Entry into the Host Cells. FEMS Microbiol. Rev. 2015, 39 (2), 155–170.

(13) Blazevic, J.; Rouha, H.; Bradt, V.; Heina, F. X.; Stiasny, K. Membrane Anchors of the Structural Flavivirus Proteins and Their Role in Virus Assembly. J. Virol. 2016, 90 (14), 6365–6378.

(14) Tan, T. Y.; Fibriansah, G.; Lok, S.-M. Capsid Protein Is Central to the Birth of Flavivirus Particles. PLOS Pathog. 2020, 16 (5), No. e1008542.

(15) Uno, N.; Ross, T. M. Dengue Virus and the Host Innate Immune Response. Emerg. Microbes Infect. 2018, 7 (1), 1–11.

(16) Chiang, W.-L.; Liu, X.; Si, S.; Li, Y.; Bengio, S.; Hsieh, C.-J. Cluster-GCN: An Efficient Algorithm for Training Deep and Large Graph Convolutional Networks. arXiv (Machine Learning), Aug, 8, 2019, 1905.07953, version 2. DOI: 10.48550/arXiv.1905.07953.

(17) Zhou, Z.; Zeng, C.; Nie, L.; Huang, S.; Guo, C.; Xiao, D.; Han, Y.; Ye, X.; Ou, M.; Huang, C.; Ye, X.; Wen, Z.; Yang, G.; Jing, C. The Effects of TLR3, TRIF and TRAF3 SNPs and Interactions with Environmental Factors on Type 2 Diabetes Mellitus and Vascular Complications in a Han Chinese Population. Gene 2017, 626, 41–47.

(18) Kao, Y.-T.; Lai, M. M. C.; Yu, C.-Y. How Dengue Virus Circumvents Innate Immunity. Front. Immunol. 2018, 9, 2860.

(19) Zhu, T.; Fernandez-Sesma, A. Innate Immune DNA Sensing of Flaviviruses. Viruses 2020, 12 (9), 979.

(20) Dayarahsa, S.; Jeewandara, C.; Gomes, L.; Somathilaka, G.; Jayathilaka, D.; Vimalachandran, V.; Wijekwrickama, A.; Narangoda, E.; Idamputtaya, D.; Ogg, G. S.; Malavige, G. N. Similarities and Differences between the ‘Cytokine Storms’ in Acute Dengue and COVID-19. Sci. Rep. 2020, 10 (1), 19839.

(21) Yakimovich, A.; Witte, R.; Andriasyan, V.; Georgi, F.; Greber, U. F. Label-Free Digital Holo-Tomographic Microscopy Reveals Virus-Induced Cytopathic Effects in Live Cells. mSphere 2018, 3 (6), e00599-18.

(22) Baer, A.; Kehn-Hall, K. Viral Concentration Determination Through Plaque Assays: Using Traditional and Novel Overlay Systems. J. Vis. Exp. 2014, 93, e52065.

(23) Kaufmann, S. H. E.; Kabelitz, D. The Immune Response to Infectious Agents. Immunology of Infection 2010, 37, 1–20.
Positively Correlates with Thrombocytopenia and Elevated Hematocrit in Dengue Infected Paediatric Patients. J. Infect. Public Health 2021, 14 (1), 1701–1707.

(29) Zhu, X.; He, Z.; Yuan, J.; Wen, W.; Huang, X.; Hu, Y.; Lin, C.; Pan, J.; Li, R.; Deng, H.; Liao, S.; Zhou, R.; Wu, J.; Li, J.; Li, M. IFITM3-Containing Exosome as a Novel Mediator for Anti-Viral Response in Dengue Virus Infection. Cell. Microbiol. 2015, 17 (1), 105–118.

(30) Oliveira, A. F. C. d.; Seixeiro, R. J.; Oliveira, A. S. d.; Souza, A. P. M. d.; Silva, M. L. d.; Paula, S. O. d. de. Potential Antivirals: Natural Products Targeting Replication Enzymes of Dengue and Chikungunya Viruses. Molecules 2017, 22 (3), 505.

(31) Falgout, B.; Pethel, M.; Zhang, Y. M.; Lai, C. J. Both Nonstructural Proteins NS2B and NS3 Are Required for the Proteolytic Processing of Dengue Virus Nonstructural Proteins. J. Virol. 1991, 65 (5), 2467–2475.

(32) Yusof, R.; Clum, S.; Wetzel, M.; Murthy, H. M. K.; Padmanabhan, R. Purified NS2B/NS3 Serine Protease of Dengue Virus Type 2 Exhibits Cofactor NS2B Dependence for Cleavage of Dibasic Amino Acids in Vitro. J. Biol. Chem. 2000, 275 (14), 9963–9969.

(33) Gong, E. Y.; Kenens, H.; Ivens, T.; Dockx, K.; Vermeiren, K.; Vandenbroucke, G.; Devogelaere, B.; Lory, P.; Kraus, G. Expression and Purification of Dengue Virus NS5 Polymerase and Development of a High-Throughput Enzymatic Assay for Screening Inhibitors of Dengue Polymerase. In Antiviral Methods and Protocols. Methods in Molecular Biology, Vol. 1030; Gong, E., Ed.; Humana Press: Totowa, NJ, 2013; pp 237–247.

(34) Chiswick, E. L.; Duffy, E.; Japp, B.; Remick, D. Detection and Quantification of Cytokines and Other Biomarkers. In Leucocytes. Methods in Molecular Biology, Vol. 844; Ashman, R., Ed.; Humana Press: Totowa, NJ, 2012; pp 15–30.

(35) Raut, C. G.; Deolankar, R. P.; Kolhapure, R. M.; Goverdhani, M. K. Susceptibility of Laboratory-Bred Rodents to the Experimental Infection with Dengue Virus Type 2. Acta Virol. 1996, 40 (3), 143–146.

(36) Chen, R. E.; Diamond, M. S. Dengue Mouse Models for Evaluating Pathogenesis and Countermeasures. Curr. Opin. Virol. 2020, 43, 50–58.

(37) Mota, J.; Rico-Hesse, R. Dengue Virus Tropism in Humanized Mice Recapitates Human Dengue Fever. PLoS One 2011, 6 (6), No. e20762.

(38) Chan, C. Y. Y. Determinants of Symptomatic Flavivirus Infection. Ph.D. Thesis, National University of Singapore, 2017.

(39) Hunsperger, E. A.; Yoksan, S.; Bucy, P.; Nguyen, V. C.; Sekaran, S. D.; Enria, D. A.; Pelegrino, J. L.; Vázquez, S.; Artibon, H.; Drebout, M.; Gubler, D. J.; Halstead, S. B.; Guzman, M. G.; Margolis, H. S.; Nathanson, C.-M.; Lic, N. R. R.; Besof, K. E.; Kliks, S.; Peeling, R. W. Evaluation of Commercially Available Anti–Dengue Virus Immunoglobulin M Tests. Emerg. Infect. Dis. 2009, 15 (3), 436–440.

(40) Guzman, G.; Dacanay, A.; Andaya, B.; Alejandro, G. Ethnopharmacological Studies on the Uses of Euphorbia Hirta in the Treatment of Dengue in Selected Indigenous Communities in Pangasian (Philippines). J. Intercult. Ethnopharmacol. 2016, 5 (3), 239.

(41) Hossain, S.; Urbi, Z.; Karuniawati, H.; Mohiuddin, R. B.; Moh Qrimita, A.; Allzrag, A. M. M.; Ming, L. C.; Pagano, E.; Capasso, R. Andrographis Paniculata (Burm. f.) Wall. Ex Nees: An Updated Review of Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. Life 2021, 11 (4), 348.

(42) Jiang, W. L.; Luo, X. L.; Kuang, S. J. Effects of Alternanthera Philoxeroides Griseb against Dengue Virus in Vitro. Di Yi Jiuan Yi Da Xue Xue Bao 2005, 25 (4), 454–456.

(43) Tang, L. I.; Ling, A. P.; Koh, R. Y.; Chye, S. M.; Voon, K. G. Response in Dengue Virus Infection. BMC Complement. Altern. Med. 2012, 12 (1), 5.

(44) Huang, N. C.; Hung, W.-T.; Tsai, W.-L.; Lai, F.-Y.; Lin, Y.-S.; Huang, M.-S.; Chen, J.-J.; Lin, W.-Y.; Weng, J.-R.; Chang, T.-H. Ficus Septa Plant Extracts for Treating Dengue Virus in Vitro. PeerJ. 2017, 5, No. e3448.

(45) Ono, L.; Wollinger, W.; Rocco, I. J.; Coimbra, T. L.; Gorin, P. A.; Sierakowski, M.-R. In Vitro and in Vivo Antiviral Properties of Sulfated Galactomannans against Yellow Fever Virus (Bel111 Strain) and Dengue 1 Virus (Hawaii Strain). Antiviral Res. 2003, 60 (3), 201–208.

(46) Taib, M.; Rezzak, Y.; Bouyazza, L.; Lyousse, B. Medicinal Uses, Phytochemistry, and Pharmacological Activities of Quercus Species. Evidence-Based Complement. Altern. Med. 2020, 2020, 1–20.

(47) Abd Rahman, N.; Hadinari, Muliawati, S.; Nor Rashid, N.; Muhamad, M.; Yusof, R. Studies on Quercus Iusitanica Extract on DENV-2 Replication. Dengue Bull. 2006, 30, 260–269.

(48) Rosmalena, R.; Elya, B.; Dewi, B. E.; Fithriyah, F.; Desti, H.; Angelina, M.; Hanafti, M.; Lotuling, P. D.; Prasasty, V. D.; Seto, D. The Antiviral Effect of Indonesian Medicinal Plant Extracts Against Dengue Virus In Vitro and In Silico. Pathogens 2019, 8 (2), 85.

(49) Brandão, G.; Kroon, E.; Souza, D.; Filho, J.; Oliveira, A. Chemistry and Antiviral Activity of Arrabidaea Pulchra (Bignoniaceae), a Potentially Useful Source of Anti-Dengue Drugs from the State of Minas Gerais, Brazil. Lett. Appl. Microbiol. 2013, 53 (6), 602–607.

(50) Ling, A. P.; Khoo, B. F.; Seah, C. H.; Foo, K. Y.; Cheah, R. K.; Chye, S. M.; Kio, I. H.; Inhibitory Activities of Methanol Extracts of Andrographis Paniculata and Ocimum Sanctum against Dengue-1 Virus. N. J. Ethnopharmacol. 2014, 155, 7919–7932.

(51) Parida, M.; Upadhyay, C.; Pandya, G.; Jana, A. Inhibitory Potential of Neem (Azadirachta Indica Juss) Leaves on Dengue Virus Type-2 Replication. J. Ethnopharmacol. 2002, 79 (2), 273–278.

(52) Sinnoe, L. R.; Macel, G. M.; Brandão, G. C.; Kroon, E. G.; Castilho, R. O.; Oliveira, A. B. Antiviral Activity of Distictella elongata (Vahl) Urb. (Bignoniaceae), a Potentially Useful Source of Anti-Dengue Drugs from the State of Minas Gerais, Brazil. Lett. Appl. Microbiol. 2013, 53 (6), 602–607.

(53) Ling, A. P.; Khoo, B. F.; Seah, C. H.; Foo, K. Y.; Cheah, R. K.; Chye, S. M.; Kim, I. H.; Inhibitory Activities of Methanol Extracts of Andrographis Paniculata and Ocimum Sanctum against Dengue-1 Virus. Int. Conf. Biol. Environ. Food Eng. 2014, 4–5.

(54) Tan, Y. P.; Xue, Y.; Savchenko, A. I.; Houston, S. D.; Modhiran, N.; McMillan, C. L. D.; Boyle, G. M.; Bernhardt, P. Y.; Young, P. R.; Watterson, D.; Williams, C. M.; Basnarals A, B, and C; Highly Oxygenated Pimarane Diterpenoids from Basilicum Polystachyon. J. Nat. Prod. 2019, 82 (10), 2828–2834.

(55) Ramalingam, S.; Karuppannan, S.; Padmanabhan, P.; Vijayan, S.; Sheriff, K.; Palani, G.; Krishnasamy, K. K. Anti-Dengue Activity of Andrographis Paniculata Extracts and Quantification of Dengue Viral Inhibition by SYBR Green Reverse Transcription Polymerase Chain Reaction. Ayu 2018, 39, 87–91.

(56) Rothan, H. A.; Zulqarnain, M.; Ammar, Y. A.; Tan, E. C.; Rahman, N. A.; Yusof, R. Screening of Antiviral Activities in Medicinal Plants Extracts against Dengue Virus Using DENV2-NS3 Protease Assay. Trop. Biomed. 2014, 31 (2), 286–296.

(57) Panya, A.; Sawadse, N.; Songkrakhon, P.; Tragoolpua, Y.; Rotarayanont, S.; Chooowongkomkorn, K.; Yenchitsomphon, M. A Synthetic Bioactive Peptide Derived from the Asian Medicinal Plant.
Acacia Catechu Binds to Dengue Virus and Inhibits Cell Entry. *Viruses* 2020, 12 (11), 1267.

(58) Hidari, K. I. P. J.; Takahashi, N.; Arihara, M.; Nagaoka, M.; Morita, K.; Suzuki, T. Structure and Anti-Dengue Virus Activity of Sulfated Poly saccharide from a Marine Alga. *Biochem. Biophys. Res. Commun.* 2008, 376 (1), 91–95.

(59) Pujol, C.; Estevez, J.; Carlucci, M.; Ciancia, M.; Cerezo, A.; Damonte, E. Novel DL-Galactan Hybrids from the Red Seaweed Gymnogongr us Torulosus Are Potent Inhibitors of Herpes Simplex Virus and Dengue Virus. *Antivir. Chem. Chemother.* 2002, 13 (2), 83–89.

(60) Lu, L. L.; Liu, Y. J.; Yang, S. G.; Zhao, Q. J.; Wang, X.; Gong, W.; Han, Z. B.; Xu, Z. S.; Lu, Y. X.; Liu, D.; C. Z. Isolation and Characterization of Human Umbilical Cord Mesenchymal Stem Cells with Hematopoiesis-Supportive Function and Other Potentials. *Haematologica* 2006, 91 (8), 1017–1026.

(61) Ng, Y.-L.; Mann, V.; Rahbaran, S.; Lewsey, J.; Gulabivala, K. Outcome of Primary Root Canal Treatment: Systematic Review of the Literature – Part 2. Influence of Clinical Factors. *Int. Endod. J.* 2007, 0, 6–31.

(62) Leardkamolkarn, V.; Sirigulpanit, W.; Kinney, R. M. Characterization of Recombinant Dengue-2 Virus Derived from a Single Nucleotide Substitution in the 5′ Noncoding Region. *J. Biomed. Biotechnol.* 2010, 2010, 1–8.

(63) Mili, B.; Ahmed, A.; Kushwaha, R. S.; Chandrul, K. K. Herbs and Herbal Therapy for Dengue. *Int. J. Trend Sci. Res. Dev.* 2019, 3 (4), 103–108.

(64) Tong, X.; Qin, H.; Zhang, X.; Shi, L.; Wang, G.; Ji, F.; Ding, H.; Tang, W.; Ding, K.; Zuo, J. WSS45, a Sulfated α-D-Glucan, Strongly Interferes with Dengue 2 Virus Infection in Viro. *Acta Pharmacol. Sin.* 2010, 31 (5), 585–592.

(65) REES, C.; COSTIN, J.; FINK, R.; MCMICHAEL, M.; FONTAINE, K.; ISERN, S.; MICHAEL, S. In Vitro Inhibition of Dengue Virus Entry by P-Sulfoxyl-Cinnamic Acid and Structurally Related Combinatorial Chemistries. *Antiviral Res.* 2008, 80 (2), 135–142.

(66) Kuo, Y.-T.; Liu, C.-H.; Li, J.-W.; Lin, C.-J.; Jassey, A.; Wu, H.-N.; Perng, G. C.; Yen, M.-H.; Lin, L.-T. Identification of the Antiviral Activity Restricting Dengue Virus Entry. *Sci. Rep.* 2020, 10 (1), 16378.

(67) TALARICO, L.; PUJOL, C.; ZIBETTI, R.; FARIA, P.; NOSEDA, M.; DUARTE, M.; DAMONTE, E. The Antiviral Activity of Sulfated Polysaccharides against Dengue Virus Is Dependent on Virus Serotype and Host Cell. *Antiviral Res.* 2005, 66 (2–3), 103–110.

(68) Abdul Ahmad, S. A.; Palanisamy, U. D.; Tejo, B. A.; Chew, M. F.; Tham, H. W.; Syed Hassan, S. Geraniin Extracted from the Rind of Nephelium Lappaceum Binds to Dengue Virus Type-2 Envelope Protein and Inhibits Early Stage of Virus Replication. *Virology* 2017, 14 (1), 229.

(69) Srirangkulk, P.; Yuttithammnon, W.; Sueroengt, A.; Pankew, S.; Hengphaatporn, K.; Rungrongmongkol, T.; Phuwprasirsiran, P.; Ruxunrundh, K.; Boonyasuppayakorn, S. A Novel Flavanone Derivative Inhibits Dengue Virus Fusion and Infectivity. *Antiviral Res.* 2018, 151, 27–38.

(70) Panraksa, P.; Ramphan, S.; Khongwichit, S.; Smith, D. R. Activity of Andrographolide against Dengue Virus. *Antiviral Res.* 2017, 139, 69–78.

(71) Lim, S. Y. M.; Chiang, J. Y.; Pan, Y. Recent Insights on Anti-Dengue Virus (DENV) Medicinal Plants: Review on in Vitro, in Vivo and in Silico Discoveries. *All Life 2021*, 14 (1), 1–33.

(72) Hishiki, T.; Kato, F.; Tajima, S.; Toume, K.; Umezaki, M.; Takasaki, T.; Miura, T. Hirsutine, an Indole Alkaloid of Uncaria Rhynchophylla, Inhibits Late Step in Dengue Virus Lifecycle. *Front. Microbiol.* 2017, 8, 1674.

(73) Whitby, K.; Pierson, T. C.; Geiss, B.; Lane, K.; Engle, M.; Zhou, Y.; Doms, R. W.; Diamond, M. S. Castanospermine, a Potent Inhibitor of Dengue Virus Infection In Vitro and In Vivo. *J. Virol.* 2005, 79 (14), 8698–8706.

(74) Kusyati, E.; N. N. Garlic Extract to Increase Platelet Levels in Dengue Hemorrhagic Fever Patients. *Heal. Notions 2017*, 1 (2), 83–85.

(75) Hall, A.; Troupin, A.; Londono-Renteria, B.; Colpitts, T. Garlic Organosulfur Compounds Reduce Inflammation and Oxidative Stress during Dengue Virus Infection. *Viruses* 2017, 9 (7), 159.

(76) Sood, R.; Raut, R.; Tyagi, P.; Pareek, P. K.; Barman, T. K.; Singhal, S.; Shirumalla, R. K.; Kanoje, V.; Subbarayan, R.; Rajerethinam, R.; Sharma, N.; Kanauja, A.; Shukla, G.; Gupta, Y.; Katiyar, C. K.; Bhatnagar, P. K.; Upadhyay, D. J.; Swaminathan, S.; Khanna, N. Cissampelos Pareira Linn: Natural Source of Potent Antiviral Activity against All Four Dengue Virus Serotypes. *PLoS Negl. Trop. Dis.* 2015, 9 (12), No. e004255.

(77) Yu, J.-S.; Wu, Y.-H.; Tsend, C.-K.; Lin, C.-K.; Hsu, Y.-C.; Chen, Y.-H.; Lee, J.-C. Schisandrin A Inhibits Dengue Viral Replication via Upregulating Antiviral Interferon Responses through STAT Signaling Pathway. *Sci. Rep.* 2017, 7 (1), 45171.

(78) Ruxrungtham, K.; Boonyasuppayakorn, S.; Hengphasatporn, K.; Rungrotmongkol, T.; Phuwapraisirisan, P.; Tham, H. W.; Syed Hassan, S. Geraniin Extracted from the Rind of Nephelium Lappaceum Binds to Dengue Virus Type-2 Envelope Protein and Inhibits Early Stage of Virus Replication. *Antivir. Chem. Chemother.* 2002, 13 (2), 83–89.

(79) Ruxrungtham, K.; Boonyasuppayakorn, S.; Hengphasatporn, K.; Rungrotmongkol, T.; Phuwapraisirisan, P.; Tham, H. W.; Syed Hassan, S. Geraniin Extracted from the Rind of Nephelium Lappaceum Binds to Dengue Virus Type-2 Envelope Protein and Inhibits Early Stage of Virus Replication. *Antivir. Chem. Chemother.* 2002, 13 (2), 83–89.
(89) Trujillo-Correa, A. I.; Quintero-Gil, D. C.; Diaz-Castillo, F.; Quinones, W.; Robledo, S. M.; Martinez-Gutierrez, M. In Vitro and in Silico Anti-Dengue Activity of Compounds Obtained from Psidium Guajava through Bioprospecting. BMC Complement. Altern. Med. 2019, 19 (1), 298.

(90) Allard, P.-M.; Leyssen, P.; Martin, M.-T.; Bourjot, M.; Dumontet, V.; Eydoux, C.; Guillemot, J.-C.; Canard, B.; Poullain, C.; Guéritte, F.; Litaudon, M. Antiviral Chlorinated Daphnane Diterpenoid Orthoesters from the Bark and Wood of Trigonostemon Cherrieri. Phytochemistry 2012, 84, 160−168.

(91) Yu, J.-S.; Tseng, C.-K.; Lin, C.-K.; Hsu, Y.-C.; Wu, Y.-H.; Hsieh, C.-L.; Lee, J.-C. Celastrol Inhibits Dengue Virus Replication via Up-Regulating Type I Interferon and Downstream Interferon-Stimulated Responses. Antiviral Res. 2017, 137, 49−57.

(92) Norahmad, N. A.; Mohd Abd Razak, M. R.; Mohmd Misnan, N.; Md Jelas, N. H.; Sastu, U. R.; Muhammad, A.; Ho, T. C. D.; Jusoh, B.; Zolkifli, N. A.; Thayan, R.; Mat Ripen, A.; Zainol, M.; Syed Mohamed, A. F. Effect of Freeze-Dried Carica Papaya Leaf Juice on Inflammatory Cytokines Production during Dengue Virus Infection in AG129 Mice. BMC Complement. Altern. Med. 2019, 19 (1), 44.

(93) Lee, Y.-R.; Yeh, S.-F.; Ruan, X.-M.; Zhang, H.; Hsu, S.-D.; Huang, H.-D.; Hsieh, C.-C.; Lin, Y.-S.; Yeh, T.-M.; Liu, H.-S.; Gan, D.-D. Honeysuckle Aqueous Extract and Induced Let-7a Suppress Dengue Virus Type 2 Replication and Pathogenesis. J. Ethnopharmacol. 2017, 198, 109−121.