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Bio-therapeutic intervention for dengue infection-present scernario on plant-based remedies: A review article

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Abstract Dengue virus (DENV), a member of the *Flaviviridae*, is the causative organism of Dengue hemorrhagic fever and Dengue Shock Syndrome. It exists as 4 serotypes (DEN1-4), all of which can cause full spectrum of the disease. Dengue is one of the most re-emerging arboviral diseases in the world with a record of 390 million cases globally, putting 3.9 billion people at the risk of infection annually. With dengue being enlisted as a high burden disease in the tropical countries, there is a dire need to bring about efficient therapeutics that help to prevent and treat the diseased population. The present mode of treatment of Dengue is based on the management of symptoms and includes the use of Non-steroidal Anti-inflammatory Drugs (NSAIDs), antibiotics, antipyretics and corticosteroids. These provide symptomatic treatment and also bring about undesirable side effects. Presently, there are no approved vaccines or antivirals for Dengue treatment in India. It is thus important to consider the biology of the virus with special focus on identification of effective anti-viral targets for therapy. With the alleviation of symptoms alone being insufficient for the disease treatment, there is a shift in research to find alternative forms of medicine for the treatment of the disease. Herbal remedies have a long history of use in ancient medical systems and are now widely accepted as safe, natural substitutes for pharmaceutical treatments. But in order to guarantee consistency and safety, the growing demand for these items calls for strict quality control methods. In particular, leaves of *Carica papaya* and Nilavembu kudineer are potential candidates for this purpose. This review focuses on bringing to light the various therapeutic interventions presently in use and emphasize the significance of quality control techniques in regulating various aspects of the development, manufacturing, and distribution of herbal products.

Keywords: Flaviviridae, NSAIDs, *Carica papaya*, Nilavembu Kudineer, Herbal technology

Introduction

Dengue is a mosquitoe borne viral disease transmitted by the vector *Aedes aegypti* and in some cases by *Aedes albopictus*. The dengue virus (DENV) belongs to the *Flaviviridae* family and the four different serotypes of DENV are DEN-1, DEN-2, DEN-3 and DEN- 4. All four serotypes have been

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reported to cause full spectrum of the disease. Dengue is enlisted as the most serious re-emerging arboviral disease (Ferreira-de-Lima and Lima, 2018) and The number of dengue infections and deaths reported in endemic areas has been rising since the beginning of 2023, and the disease has also spread to formerly dengue-free areas (WHO, 2024). Dengue is described as an alarming public health threat in countries of tropical Asia and Latin America (Deen, 2016) among which India is also included as a high endemic country. The classification of disease caused by dengue infection is described as (i) Dengue with or without warning signs for progression towards severe dengue, characterized by persistent vomiting, mucosal bleeding, increase in haematocrit and decrease in platelet count and (ii) Severe dengue whose symptoms include severe plasma leakage, respiratory distress, severe internal bleeding and severe organ impairment. The most pronounced symptoms of dengue include the dengue hemorrhagic fever, which progresses to thrombocytopenia with the patient experiencing hemorrhagic manifestation and bleeding through nose, gums and gastrointestinal tract primarily due to the increased vascular permeability and plasma leakage. These symptoms would further develop into circulatory compromise, dengue shock syndrome and ultimately result in death (Gowda *et al.,* 2015). With dengue becoming a high burden disease in the tropical countries, there is a dire need to bring about efficient therapeutics that can either help to prevent or treat the disease population. The treatment of dengue is presently based on the clinical manifestations of dengue illness and hence the various therapeutic interventions presently (Figure 1) existent must be focused on developing vaccines and antivirals to combat severity of dengue.

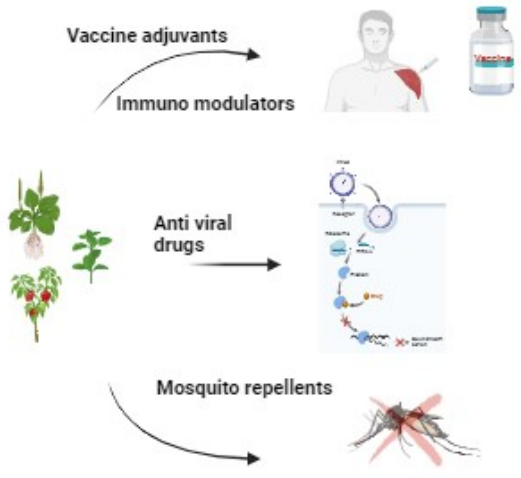

Figure 1. Therapeutic interventions for Dengue infection

Biology of DENV- understanding the antiviral targets

The envelope (E) protein and the membrane (M) protein, which are both derived from the precursor prM, make up the two outer membrane proteins of the enveloped virus DENV. The genome is thought to be wrapped/associated with the capsid protein (C). The DENV genome encodes a single polyprotein which is cleaved by serine proteases, NS2B and NS3, along with the seven non-structural proteins. The seven non-structural proteins (NS1, NS2A, NS3, NS4A, NS4B, NS5) also perform an added function of genome replication and capping. A few of these NS proteins also take part in pathogenesis and act against the host cell's innate defenses. Within the cytoplasm, DENV replicates. (Bulich and Aaskov, 1992However, DENV virus multiplication is facilitated by the translocation of the capsid protein (C) to the nucleoli of infected cells (Wang et al., 2002; Iglesias et al., 2015). The DENV C is a small (~12 kDa), highly basic protein with three nuclear localization signals (NLS) encoded at the 5' end of Flavivirus genome (Wang *et al.,* 2011). E protein is involved in dual functions by recognizing the cellular receptor DC-SIGN, L-SIGN, the high affinity laminin receptor, the mannose receptor, and GRP78. It is also involved in fusion of viral membrane to cellular endosomic membranes. These M and E structural proteins have been considered to be promising drug targets so far. When antivirals are targeted against E protein, it would impede the binding and spreading of virus to target cells before infection. Out of the seven Non-Structural proteins, only NS3 and NS5 have been explored as potential therapeutic targets so far. This is due to their dual roles in virus replication and their demonstrated enzymatic activity, which is highly advantageous for drug screening. On the other hand, a NS2B/NS3 protease has been the first dengue protein targets actively used in drug design programmes. Examination of the cellular targets for anti-dengue research revealed that the host cell is actively involved in many levels during DENV infection. This involvement could be either at the level of innate immunity and counteraction thereof, or providing co-factors and template for replication of the virus. When intraperitoneal and intranasal immunization are administered simultaneously, functional antibodies (anti-DIIIC and anti-DENV) and an in vitro response of IFN-γ secretion are generated (Sangiambut et al., 2008). Hence, theoretically, any of the cellular proteins involved in DENV life cycle may be a potential target for antiviral therapy. The strategy may differ depending upon whether the cellular protein must be activated or inactivated. Exploration of non-nucleoside inhibitors and inhibitor ligands as the drug targets must be done by knowing the fact that the DENV genome is constantly prone to mutations. A screen of 120 kinase inhibitors resulted in the discovery of the anti-dengue effect of dasatinib, a known c-Src kinase inhibitor. Targets being caught in the process of performing their vital enzymatic functions include the DENV polymerase and protease, and these may be extremely helpful in the production of designer compounds that

may serve as effective inhibitors. (Lazo *et al.,* 2017). Both antibodies and T-cell response contribute to protection and clearance of DENV infection. Neutralizing Abs have been shown to play a significant protective function in the process of DENV infection, reduction in DHF/DSS during early infancy less than 6 months, and passive Abs transfer trials in animals (Low et al., 2017). The discovery of epitopes recognized by potent neutralizing mAbs, following the natural DENV infection, has important implications for dengue vaccine development. Strong neutralizing epitopes identified by human mAbs during secondary infection include DIII, the hinge region of DI/II, and quaternary epitopes on virion, as well as the E-dimer epitope and fusion loop epitope. The identification of epitopes recognized by mAbs that are weakly or non-neutralizing and enhancing DENV in FcγR such as anti-prM antibodies, may also be considered as prospective candidates (Murphy and Whitehead, 2011).

Several siRNA are involved in interacting with and inhibiting the viral replication cycle. But for use of siRNA as a therapeutic, their efficacy needs to be demonstrated. The viral-cellular protein interactions or interaction inhibitors that have to be discovered will constitute as targets to either control viral growth or pathogenicity. The anti-dengue drugs including direct antivirals, host modulators, and RNAi therapeutics have also been investigated (Tsai *et al.,* 2017). With respect to medicinal plant derivatives, a significant amount of research has been dedicated to hypothesis–driven and practice-based naturally occurring compounds possessing anti-dengue properties (Koishi *et al.,* 2012; Tang *et al.,* 2012; Abd Kadir *et al.,* 2013; Lee *et al.,* 2013; Dn and Rt, 2014 and Padilla-s *et al.,* 2014). Regardless of whether they are evaluated for drug development, several of the plant derivatives are already being used to treat dengue in conventional settings, which emphasizes the need for further investigation at their impact on dengue-related outcomes. Major challenges in the process will include identification of compounds that can be validated *in vivo* (Ahmad *et al.,* 2011), confirmation of safety profile, activity beyond the earliest hours of infection, cost and viral resistance.

A study on alternative antiviral strategies including Bromocriptine inhibition of translation or RNA synthesis in DENV life cycle has been conducted by drug addiction and drug elimination assays (Wang *et al.,* 2011). In order to effectively decrease the number of infected mosquito vectors and hence restrict the spread of DENV in the wild, mosquito-based dengue immunization techniques have also been developed (Chan *et al*., 2017).

Recently, NITD-688 have been identified as pan serotype chemical inhibitor of NS4B by high throughput phenotypic screening against 4 serotypes. It was proved oral efficacy in infected AG129 mice when treated with 30 milligrams per kg for twice a day. The log reduction value was 1.44 and 1.16 when given in time of infection and 48 hours infection mice. *In-vitro* toxicology have proved long elimination half-life and good oral bioavailability in 7 days repeat tolerance test.

SAR exploration on spiropyrazolopyridone core inhibition at 3' or 5' site on DENV-2 and DENV-3 potency. JMX0376 and JMX0395 with 2,4-bis(trifluoromethyl) benzyl inhibitory activity in DENV 1 – DENV 3.

JNJ-A07 inhibitor blocks NS3-NS4B binding and blocks the viral replication. In this study 21 clinical isolates of natural genetic diversity mouse models were involved. Cell-based anti-DENV2 was proved pan-genotype and pan-serotype in JNJ-A07 inhibitor (Kocienski, 2021; Moquin *et al.,* 2021; Li and Kang, 2022 and Xu *et al.,* 2020).

Challenges for Dengue treatment

The presently available method for the treatment of dengue is purely symptomatic; the patient is required to be transfused with platelets and fluid management by scrupulous monitoring of the blood parameters. If the immunity develops against the infection, it is only protective to the same serotype; a secondary infection by a different serotype may result in more severe symptoms (Liu *et al.,* 2016) during which no neutralizing antibodies are produced. These non-specific antibodies to one serotype bind to virions of another serotype rather than neutralizing them. These complexes of antibodies bound to virions, when presented to the T-cells, induce cytokine production and leads to endothelial dysfunction. This leads to the display of the most obvious symptoms, i.e., increased capillary permeability and vascular leakage. The DENV thereby exhibits molecular mimicry with endothelial or platelet cells, to which cross reactive anti-dengue virus antibodies are bound (Huang *et al.,* 2014). In other words, there is no long-term cross-protective immunity. Till date there are no approved effective vaccines or antiviral drugs available for Dengue in several countries including India. Consequently, a balanced immunity against all four DENV serotypes is necessary for a vaccine to be effective. Such challenges of dengue vaccines need to be overcome to meet the urgency for antiviral development. Also, it must be noted that the four serotypes of dengue are antigenically diverse and such complexity contributes to the challenge in formulation of dengue vaccines (Kudineer, 2013). A study at the molecular level indicates that there are certain genes that influence the production and aggregation of platelets- the *Arachidonate-12-lipoxygenase (ALOX12)* gene and the *Platelet Activating Factor Receptor (PTAFR)* gene (Vannice *et al.,* 2016). The *ALOX12* gene has been found to have a high expression in megakaryocytes, which are responsible for proper production of platelets. On the other hand, *PTAFR* gene, which is also expressed in megakaryocytes, has been postulated to possess the dual function of being a precursor for platelet production as well as aiding in platelet aggregation. Further investigations in the expression levels of these genes in dengue affected patients could help explain the mechanism, by which DENV causes the symptoms and the underlying reason behind the same.

Immunization strategies & challenges for vaccine development

An ideal dengue vaccine should be able to produce a life-long protective immune response in the form of neutralizing antibodies that are able to be effective against all the four serotypes of dengue virus (DEN1-4). The concern of eliciting equal protection against all dengue virus serotypes is crucial because of the phenomenon of antibody-dependent enhancement (ADE). High titers of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies develop during primary infection at 3-5 and 6-10 days after the infection begins, respectively. The presence of IgM is transient, disappearing 2-3 months after the onset of illness, whereas IgG persists for life (Halstead *et al.,* 2010). Hence, primary infection with a particular serotype provides life-long immunity against that serotype. However, against the remaining serotypes, it does not offer continuous cross-protective immunity.

It's called the ADE phenomena. At low concentrations of antibodies against primary infection, it is discovered that these cross-reactive antibodies that were produced against the fusion loop and prM are weakly neutralizing and accelerate infection (Guzman *et al.,* 2010). ADE, however, poses disease severity in addition to humoral immunity. Also, cross-reactive memory T cells could play a role in either providing protective immunity or causing immunopathology. Another impediment to the process of vaccine development for Dengue is the lack of animal models. The animal models are useful to elucidate the pathogenesis, immune response and clinical course of dengue infection in humans. If the mouse-adapted DENV strain is inoculated into suckling mice, it causes paralysis and death. Severe combined immune-deficient (SCID) mice engrafted with human carcinoma or dendritic cells, are unsuitable for studying immune responses in dengue vaccine pre-clinical trials (An *et al.,* 1999; Johnson and Roehrig, 1999 and Dejnirattisai *et al.,* 2010). Again the risk of developing severe dengue due to ADE during secondary infection after vaccination cannot be evaluated using non-human primate (NHP) models (Lin *et al.,* 1998). With respect to evaluation of dengue vaccine candidates, AG129 mouse model has been recommended by WHO. It should be noted that this model allows limited evaluation since it lacks both type I and type II IFN pathways. Hence, this limits production of high titre neutralizing antibodies, which may further result in ADE (Perng *et al.,* 2011). The drawback of plaque reduction assay using cell lines like Vero or LLC-MK2 is that they lack surface expressed Fcγ receptors. Since cells of monocyte and macrophage lineages (targets for DENV replication in human body) abundantly express Fcγ receptors, which allow DENV entry into these cells, immunodiagnostic assays performed on cells lacking Fcγ receptors may not be true predictors of protective immune response (Jin *et al.,* 2009; Moi *et al.,* 2012 and Zellweger and Shresta, 2014). Additionally there is no standardised immunoassay to measure T-cell-mediated immune response to non-structural proteins during a

heterologous DENV infection. Measuring T cell response is found to be significant because of the mounting of memory $CD8^+$ T-cell response to the non-structural antigen-3 (NS-3) of a DENV during secondary infection, which leads to stimulation of inflammatory response by release of tumour necrosis factor-α (TNF-α), a key feature of DHF and DSS (Jin *et al.,* 2009; Sun *et al.,* 2009 and Whitehorn and Simmons, 2011). It must also be noted that the research for anti-viral activities in the laboratory poses to be cost enduring. There is a need for RT-PCR for the diagnosis of Dengue serotypes or antigen detection in all vaccines with febrile illness. Despite the existing challenges for an ideal dengue vaccine, development of dengue vaccine candidates through various approaches has been progressed and field trials of these vaccine candidates are also being conducted (Table 1).

Replicating viral vaccines

Current methods of producing live-attenuated viruses for dengue vaccines include attenuation by serial passage in cell lines and targeted mutagenesis and by constructing chimeric vaccine viruses. Advantages of these methods include robust nature, long lasting and broad immunity as well as lower production cost. Disadvantages include difficulty in attenuation, genetic instability, possibility of reversion, and interference in the case of multicomponent LAV vaccines (Khetarpal and Khanna, 2016).

Non-replicating viral vaccines

These vaccine candidates are not capable of replicating and thus offer the advantage of conferring immunity without the risk of infection. There are multiple strategies to develop this class of vaccines like DNA vaccines, subunit proteins, virus-like particles (VLPs), and so forth. Advantages of these methods include reduced reactogenicity, better suitability for immuno-compromised individuals, and balanced immune response in case of tetravalent formulation. There are however, certain disadvantages including less broad, potent, and durable immune responses, which may result in ADE. It may also require the use of adjuvants. The current status of classification and details of dengue vaccine candidates are summarized in Table 1. WHO recommends development of an alternative dengue vaccine candidate designed to elicit strongly neutralizing antibodies in absence of cross-reactive, enhancing antibodies. Such a vaccine candidate would enable higher efficacy and applicability to a broader group of subjects including infants and naive population.

Table 1. Current status of Dengue vaccines

Sl.No	Type of Vaccine	Clinical trial stage	Company
01	Live attenuated vaccine- Denvaxia (Hadinegoro *et al.,* 2015)	Phase II Completed and Licensed	Sanofi Pasteur
02	Live attenuated vaccine -Den Vax (Biswal *et al.,* 2020)	Phase III	US CDC/Inviragen/ Takeda
03	Live attenuated vaccine -TetraVax-DV-TV003 (TV003) (Whitehead *et al.,* 2017)	Phase III	NIAID
04	Subunit vaccine - V180 (Manoff *et al.,* 2019)	Phase I	Merck Sharp & Dohme Corp.
05	Purified in activated vaccine- TDEN-PIV (Lin *et al.,* 2021)	Phase I	U.S. Army Medical Research and Materiel Command (Walter Reed Army Institute of Research
06	DNA Vaccine- Tetravalent Dengue Vaccine (TVDV) (Porter *et al.,* 2012)	Phase I	U.S. Army Medical Research and Materiel Command (Walter Reed Army Institute of Research)

In the present scenario, Sanofi-Pasteur's CYD-TDV, commonly known as Dengvaxia was the first vaccine to be commercially available by the year 2015. The vaccine was granted approval for use on individuals 9 to 60 years of age. The license was granted in Dengue-endemic areas in 11 countries, including Paraguay, El Salvador, Mexico, Brazil, Costa Rica and Philippines. The vaccine is made of four chimeric viruses, in which the pre-membrane and envelope structural genes of the attenuated yellow fever 17D vaccine strain have been replaced with corresponding genes from each of the four dengue serotypes. Since the dengue virus and the yellow fever virus have the same genus, the live attenuated yellow fever virus was genetically engineered to possess genes encoding dengue proteins, which would in turn induce the human system to produce anti-bodies against all four serotypes of dengue. Prior to approval of the vaccine, the Phase III trials were conducted on a large scale in 5 Asian countries (on 2 to 14 year olds) and in 5 Latin American countries (on 9 to 16 year olds) and a follow up for 25 months after the first dose was monitored. Although the overall efficacy against symptomatic dengue was 60%, a statistically significant increase in the risk of hospitalized dengue was observed in the vaccinated Asian children who were within the age group of 2-5 years (Ferreira-de-Lima and Lima, 2018). This came to light when public vaccination programmes were rolled out in countries like Brazil and Philippines; the Philippines Department of Health came out with observations that the vaccination may be ineffective or may theoretically even increase the future risk of hospitalized or severe dengue illness in those who are sero-

negative at the time of first vaccination regardless of age. This meant that it was effective only in the individuals who were already affected by at least one dengue serotype and that it posed a great risk to those who have not had any infection by any of the 4 serotypes, prior to vaccination. Owing to such speculation, the clearance for Dengvaxia was suspended in Philippines in January 2018 and WHO clarified its stance to mention that Dengvaxia may be used only on those who have had a prior infection by dengue and has recommended its restricted use. The cancelling of license in Philippines has led to other countries reconsider their position with respect to implementation of the vaccine in the public domain. Nevertheless, other vaccine candidates are being evaluated for their efficacy and safety in the clinical trials. Among them, the candidates from GSK, MERCK, US NMRC, US WRAIR, US NIH and Takeda have reached various phases of the Clinical trials (Vannice *et al.,* 2016). A tetravalent formulation of recombinant lipidated dengue envelope protein domain III was proven to be a potential vaccine candidate against dengue virus (Chiang *et al.,* 2016). A DENV NS protein called NS1 protein was secreted into the extracellular milieu after vaccination, and it proved successful in reducing DENV-induced vascular leakage and severe clinical symptoms. The NS1-based immunization may be the best option that can be used in the future for developing a novel dengue vaccine since it circumvents the antibody-dependent enhancement and cross-reactive T cell-related issues brought on by current vaccination techniques. Based on recent phase III trial evaluation of the vaccine CYD-TDV found to be safe and it proved its ability to partially protect the children and adolescents against DENV 1-4 serotypes for a period of one year. The study comprised of randomised controlled trials involving children aged 2–17 years with an overall efficacy of 60%, but it varied depending upon the serotype. Long-term analyses and further research was recommended for better vaccine efficacy (Rosa *et al.,* 2019). Review of the use of vaccine was attributed to the increased risk of severe dengue virus (DENV) disease among DENV-seronegative vaccines (Sridhar *et al.,* 2018) caused by Dengvaxia (CYD-TDV) vaccine. The vaccine showed loss of efficacy among young children within 3 to 4 years after Dengvaxia immunization as a result of loss of protective antibody response implying inadequate neutralizing antibodies and excessive T-cell–mediated immunopathology (Slifka and Amanna, 2018). TetraVax-DV (LAV) sponsored by Butantan phase III trial is also expected to end on December 2022.

Efforts for dengue vaccine development have faced multiple challenges, including the need to induce a balanced and lasting immunity against four DENV serotypes; uncertainties exist around immune reactions with respect to protection against disease, potential immune enhancement of disease, and lack of suitable animal model to study immune responses. However, considerable progress has been made in recent years, which has resulted in an advanced dengue vaccine pipeline. In order to be effective against dengue, a vaccine must

be able to elicit antibodies that are mostly serotype-specific to DENV and operate as a protective barrier when serotype cross-reactive antibodies act as a disease improver. Present vaccine candidate TAK-003 (Takeda) is a live attenuated DEN-2 virus which comprises all four types of dengue strains, the study trials done at Asia-Pacific region and Latin America where the dengue is endemic. The efficacy, safety, and immunogenicity of this vaccine TAK-003 evaluated in large scale phase 3, randomized clinical trial concerning children and adolescents 4 to 16 years of age and concluded the vaccine was efficacious against virologically confirmed dengue fever (Efficacy, Safety and Immunogenicity of Takeda's Tetravalent Dengue Vaccine in Healthy Children (TIDES)) (Biswal *et al.,* 2019).

The world is awaiting an effective dengue vaccine to counter the burden it brings on the world's health. But for the present, more effective therapeutic strategies based on developing better immunogen design with DENV envelope and precursor membrane proteins, as a vaccine candidate on subsequent natural DENV infection will bring down the number of deaths caused by dengue.

mRNA Vaccine

An mRNA based vaccine against DENV has not been successfully implemented, although it has been reevaluated in light of the recent success of the mRNA vaccine against SARS-CoV-2 (Park *et al.,* 2022). The primary target Antigenic gene for an mRNA vaccine against DENV are NS3, NS4b, and NS5, which lack ADE induced by Antibodies and can activate CD8+ T cells (Roth *et al*., 2019). More studies are being conducted to produce neutralizing Abs with NS1 and 80% of E protein (E80) (Zhang *et al*., 2020). Furthermore, research is being done on creating an mRNA vaccine utilising the prM and E proteins of DENV1 (Wollner and Richner, 2021; Wollner *et al.,* 2021). The ADE was decreased by immunization with the modified fusion loop epitope in constructs including both wild-type and mutant fusion loop epitopes. Comparing the mRNA vaccine against DENV in early stages to other mRNA vaccines, it remains in its early stages; yet, the mRNA vaccine platform can be used to effectively employ NS proteins with CD8+ T cell epitopes against Flaviviridae. As a result, the development of mRNA vaccines to combat DENV is anticipated soon.

Dengue therapeutics

There are presently no effective or approved anti-viral agents for the treatment of dengue because the mechanism by which the DENV causes these symptoms is not completely understood. Nevertheless, the overall antiviral therapy is based on infected cell assay method and knowledge-based method centred on nucleoside and replication-based RT inhibitors. Knowledge based

method associated with characterization at the molecular and sometimes the atomic levels provide the particular target for the mechanism of action. Anti-viral drug targets may exist in natural sources. However, on a larger scale, to treat the symptoms such as high fever, severe headache and rash, antibiotics, and NSAIDS as well as corticosteroids are being prescribed. Alternatives to these drugs are being sought since they do not efficiently improvise the condition and worsen the symptoms to cause gastritis and bleeding (Abd Kadir *et al.,* 2013). Corticosteroids are being reported to have immune suppressing property, which further weakens the affected individual from fighting the infection. Many *in vitro* studies (Basu *et al.,* 2008; Putintseva *et al.,* 1986 and Azeredo *et al.,* 2015) have reported that the thrombocytopenic activity of the virus could be attributed to its ability to inhibit *in vitro* megarkaryopoiesis and differentiation of megakaryocytic progenitors and induce apoptotic cell death in them. Other studies suggest that the infection by DENV could trigger an autoimmune response, where antibodies produced against it by the host, and could bring about destruction of platelets. Although thrombopoietin agonists such as Eltrombopag and Romiplastim are available to boost the platelet count (Gowda *et al.,* 2015), a recent shift in the method for treatment of dengue has been observed in India. The methods of complementary and alternative medicine such as Siddha and native medicinal formulations are being preferred. In the native medicinal scriptures, the symptoms of dengue and the effective medicinal plants for cure have been clearly elucidated. There are many medicinal plants to be used to the treatment for patients infected by dengue, which have however been reported to possess properties that counteract the symptoms caused by DENV. The plant extracts of *Queruslusitanica* (gall oak) and *Gastrodiaelata* (Japanese orchid) have been demonstrated to have anti-DENV-2 activity by inhibiting the replication of the virus (Abd Kadir *et al.,* 2013. Despite the reports of various other plants possessing anti-DENV properties, the most commonly prescribed medicines are *Carica papaya* (Papaya) leaf extracts and Nilavembu kudineer. They have been demonstrated to have anti-pyretic, anti-dengue as well as anti-inflammatory effects in dengue affected patients (Abd Kadir *et al.,* 2013).

Control of arboviral vector

Apart from the use of efficient therapeutics for the treatment of dengue affected patients, it is imperative to prevent and control the population of the Dengue vector as described by Barde *et al*. (2018). DENV is transmitted from infected individuals to healthy individuals, predominantly through a bite of the mosquito *A. aegypti*. After a healthy individual is bitten by a vector, the incubation period for the virus to proliferate in the host is about 4-7 days, after which the symptoms begin to appear. It is thereby evident that, apart from treatment of individuals affected by dengue, the major challenge lies in

controlling the population of the vector that is responsible for transmitting the causative organism. Mosquito-based dengue immunization strategies have also been developed to interrupt the vector competence to effectively reduce the number of infected mosquito vectors and, thus, control the transmission of DENV in nature (Lin *et al.,* 2016). Many naturopathic approaches have been highlighted to the public such as fumigation of *Azadirachta indica* (Neem) leaves and *Eucalyptus citriodora* (Eucalyptus) leaves, whose essential oils have been shown to be natural repellent against *A. aegypti*. Camphor, extracted from *Cinnammonum camphora* as well as the active component called methyl chavicol from *Ocimum kilimandscharium* (basil) have also depicted similar anti- *A. aegypti* properties (Macaulay *et al.,* 2007). Other plant extracts such as those from *Cassia fistula* and spinosad (which is a bioproduct produced by the actinomycete *Saccharopolyspora spinosa*) have been reported to possess anti-larvicidal properties against the larvae of *A. aegypti* (Basu *et al.,* 2008). The essential oils from the reported plants are potent mosquito repellents; however, their high volatility must be taken into account. In addition to the use of plants as vector repellents, the need to maintain cleanliness and prevent water stagnation, proper waste management and disposal methods help to pave way for the prevention of vector borne diseases. Other measures such as local awareness programmes and routine door-to-door scrutiny by trained volunteers to check for mosquito larvae accumulation in and around household premises are elaborately pursued as well.

Herbal based therapeutics

Plants are important medical resources, and the natural compounds obtained from them are crucial to the treatment of many different kinds of diseases. According to a World Heath Organization report, more than 80% of the world population presently relies on traditional herbal medicines. Over 80% of the world's population currently uses traditional herbal remedies, according to a World Health Organization analysis. Its demand is projected to be between 62 and 120 million USD globally, and by 2050, it would reach 7 trillion USD. Since the majority of very valuable medicinal plants are harvested from forests, there may be a risk to the extinction of significant wild species. The production of medicinal plants is a difficult endeavor due to the paucity of knowledge regarding the biology of their seeds and agricultural techniques. To obtain notable production and quality, high-quality planting material must be developed using an approach that is scientific in nature.

Plant-based raw materials make up more than 90% of the formulations used in the Indian Systems of Medicine, which include Ayurveda, Siddha, Unani, and Homoeopathy (AYUSH). Therefore, the use of authentic, high-quality raw materials and standardized substances in the production of raw materials for the medicines is a major factor in their efficacy. AYUSH

medications are made from roughly 2000 medicinal plants, 500 of which are used more frequently than the rest. Herbs and medicinal plants have been found in the forests for millennia. In recent decades, there has been a resurgence of interest in AYUSH systems, which has led to a demand for herbs and plants, despite the uncertain availability of medicinal plants obtained from forests. As a result, the demand for medical plants and herbs is greater than the supply of forest resources, which are already under constraint. Due to irresponsible forest plant gathering practices and a lack of culture, several species are now at risk of becoming extinct. In order to ensure that medicinal plants are utilized effectively and simultaneously conserved, efforts must be taken to encourage the growth of these plants and raise public awareness, especially among farmers, of their economic and medicinal value. Good cultivation and harvesting techniques are required (Babu *et al.,* 2016).

So far, medicinal plants have been harvested from natural resources. Nevertheless, there are numerous issues with adulteration and misidentification in the plant material that was obtained from these sources. Additionally, it's possible that any or all of the other species have contaminated the plant material that was taken from the wild. The presence of the active ingredients varies across the wild types depending on the location. Any of these scenarios could have adverse consequences. Considering this, the only way to obtain raw materials of the necessary quality may be through the growth of real, original plant varieties. It has never been simple or profitable to cultivate these plants, though. They are exploited from wild sources primarily for this reason. Some major obstacles are the lack of appropriate methods, soil, and real planting material. Various factors, such as genetic makeup, ambient circumstances, collecting and growing procedures, harvest and post-harvest processing, shipping and storage practices, and so on, affect the safety and quality of raw medicinal plant components and finished products. An essential step toward ensuring the quality of herbal medicines and environmentally sound production techniques is the creation of WHO recommendations on GACPs for medicinal plants.

Organic manufacturing of herbal plants

A strict regulation should be implemented for the production of medicinal plants for the purpose of extracting pure components or as a pure raw material for phytopharmaceuticals.

With very few conditions, it should be illegal to use insecticides and herbicides. Thus, mechanical weed removal takes the place of herbicides. A standard good agricultural and collection practice (GACP) should be framed with the help of national regulatory bodies and International Organic forum like Association of Agricultural Technology in Southeast Asia (AATSEA). These species' existence will eventually be threatened by the habit of gathering wild

herbs for the commercial manufacture of traditional medicines. Planting these herbs commercially is the only way to stop the increasing demand for these herbal medications from leaving herb species in risk. A comprehensive understanding of these crops' growth requirements are crucial to ensuring that these herbs may be produced economically without losing their medicinal qualities. These include knowledge of their native growth environment, methods of collection, planting material propagation, harvesting, and management of the herbs after harvest. Since each herb species has unique growth requirements, their preferred ecological zones are very particular. Environmental factors that affect growth include temperature, moisture content, solar radiation intensity, and carbon dioxide concentrations.

The specific phytochemicals found in each species as well as the species' ability to survive will depend on this combination of variables. Certain herbs can only be found at higher elevations because they demand cooler day and nighttime temperatures. Some are shade-loving plants that thrive in the forest's underbrush. Others need direct sunshine, thus they are typically found in wide spaces, such as at the sides of roads or vacant ground. A basic understanding of plant propagation methods is necessary to provide the fastest possible delivery of high-quality materials in huge quantities. The type of plant to be utilized determines the appropriate approach. Some herbs are simple to produce from freely obtainable seeds. Certain herbs require specific seed treatments in order to ensure the production of consistent, high-quality seedlings. Certain herbs can be propagated vegetatively using rhizomes, tubers, shoot cuttings, or stolons (suckers).

System of commercial production

There are various systems for planting and producing herbs. These include open-field monoculture, integration with well-established tree crops like oil palm and rubber, and agro-forestry production methods (virgin forests, forest plantations, and cleared forests). However, it's crucial to choose which herbs to produce using various production methods. These herbs require the same agronomic practices as other well-known commercial crops. Appropriate fertilization, water management, cultural techniques, and pest control are the fundamentals of agronomy. However, some form of organic farming or biodynamics should be implemented to guarantee that the plants are grown in conditions that are most similar to their natural habitats. This includes controlling plant diseases and pests as well as using the proper non-chemical fertilizers.

Herbal remedies for Dengue

Many studies have been conducted to verify that the leaf extracts of *C. papaya* have a positive effect on the process of treatment and recovery from the infection. The methanolic leaf extracts have been demonstrated to possess *A. aegypti* larvicidal properties and contain biologically active compounds such as chymopapain and papain, which are known to increase the anti-oxidant power in blood, steroids, saponins, cardiac glycosides, phenolics and flavonoids (Bordia *et al.,* 1997). The papaya leaf extracts have been established as medicines that boost haematopoiesis and thrombopoiesis of patients under the dengue infected condition (Dharmarathna *et al.,* 2013). The mechanism by which they are able to bring about these effects could be due to their ability to inhibit destabilisation of the plasma membrane (Ranasinghe *et al.,* 2012; Pangtey *et al.,* 2016). The leaf extracts are also able to inhibit heat induced and hypotonicity induced haemolysis of erythrocytes of affected patients. The prevention of stress induced destruction of plasma membrane by the leaf extracts might be due to the presence of flavonoids, such as kaempferol, quercetin and coumaric acid Pangtey *et al.,* 2016). Based on the promising effects that the papaya leaf extracts had on the treatment of affected individuals, it has been developed into a capsule as well as a syrup by the Indian company PhytoSpecialities Pvt. Ltd. The company has brought out the product named Thrombup, which is predominantly composed of the papaya leaf extracts, into the market and it appears to be an essential cure for thrombocytopenia, where Thrombup aims at helping the platelet count.

Nilavembu kudineer, which is a concoction of 9 medicinal plants that is prepared as a concentrate, is also a prominently used Siddha medication for treatment of Dengue in India administered at doses determined by a Siddha doctor. The components of Nilavembu kudineer are *Andrographis paniculata*, *Trichosanthes cucumerina*, *Santalum album*, *Zingiber officinale*, *Cyperus rotundus*, *Vetiveri azizanioides*, *Piper nigrum*, *Mollugo cerivana* and *Cymbopo gondistans*. Each of these plants has been reported to possess various medicinal properties individually. *A. paniculata* has been demonstrated *in vitro* to enhance the proliferation of human peripheral blood lymphotcytes. It also induces the production of IL-2, thereby stimulating the T regulatory cells in combating the infective agent. *T. cucumerina* has been proven to possess anti-inflammatory properties, which attribute to the stabilization of the biological membranes and nitric oxide inhibitory activity (Arawwawala *et al.,* 2010). *S. album* has displayed modulatory effect of cellular Glutathione-S-transferase, which is known to have anti-oxidant and *in vitro* antiviral activity against HSV-1 & HSV-2 (Benencia and Courreges, 1999). A natural inhibitor of platelet aggregation would be *Z. officinale*, which possesses anti-oxidant activity in addition to its anti-atherosclerotic effect (Kim *et al.,* 2005). Anti-inflammatory property contained in medicinal plants may play a great role in tackling the

viral infection because phagocytic cells produce a large amount of hydrogen peroxide, resulting in an increased inflammatory reaction and releasing free radicals that cause cellular damage. *C. rotundus* and *V. zizanioides* have demonstrated for free radical scavenging activities (Kilani *et al.,* 2005; Meghwal and Goswami, 2013), which could account for their role in the Nilavembu kudineer mixture. *P. nigrum*, a very widely used medicinal plant, is well known for its detoxifying property, ability to enhance absorption and bioavailability of drugs and anti-oxidant property. Both *P. nigrum* and *M. cerviana* have anti-inflammatory properties (Sadique *et al.,* 1987). In addition to this, *M. cerviana* has depicted human RBC stabilizing effects *in vitro* (thereby inhibiting hemolysis) (Zhang *et al.,* 2011). The oil of *C. distans* has active components called citronellol and trans-geraniol, which have been reported to act as mosquito repellants (Cleason *et al.,* 2000).

Some Siddha experts prescribe a combination of both Nilavembu kudineer and Adathodai kudineer. Adathodai Kudineer consists of a mixture of *Adhatoda vasica* and *Borassus flabellifer*, whose leaves have been reported to possess antipyretic property and prevent haemorrhage (Paschapur *et al.,* 2009) and anti-inflammatory properties (Vasudevan *et al.,* 1999), respectively. This combination has been demonstrated to be safe among the paediatric groups, which contribute to a major portion of the dengue infection cases.

Conclusion

Dengue virus, manifesting in four serotypes, has become a challenging target to design effective drugs. Currently, there is no effective vaccination or antiviral drugs available against it. The only mode of treatment is symptomatic or preventive and supportive care. Hence, there is a strong need to develop therapeutic approaches that can prevent the progress of DENV replication and decrease viral load. One of these factors could also be eradication of the vectors, which could also lead to greatly decreasing the transmission. There are multiple leads for antiviral design advancing through the therapeutic development pipeline, and clinical trials are in progress while many more are beginning. Among existing methods of treatment, *C. papaya* leaf extracts showed promising results in the improvisation of blood parameters in dengue affected patients and has been characterized for its active components. It has also been made commercially available as a tablet and syrup to the public. Nilavembu kudineer, is a mixture that produces a synergistic effect of all the medicinal plants in it. If the mechanism, by which the DENV is able to cause the infection and related symptoms, is clearly established, targeted drugs can be developed to counteract the effect of the virus. Presently, we know that the Nilavembu kudineer and *C. papaya* leaf extracts are aiding the process of recovery for patients affected by dengue. However, it is important to outline the function of every component in the mixture so that their role as a therapeutic

agent is clearly defined, for more efficient and targeted drug discovery for the treatment of dengue. It is also necessary to address the fact that more strict regulations need to govern the process of prescription of dosage and formulation of the medication for dengue treatment. Native medicines also have a dose dependent activity and/or toxicity on the human body and these aspects also need to be highlighted and taken into account for effective treatment and rapid recovery. Ensuring the effectiveness and safety of herbal medication products through quality control is crucial for protecting consumer health, providing dependable treatment alternatives, adhering to regulatory requirements, and promoting the expansion of the herbal medicine sector.

Quality control is essential for maintaining the efficacy and safety of herbal medicine products, safeguarding consumer health, offering dependable treatment choices, complying with regulatory requirements, and encouraging the growth of the herbal medicine industry. Furthermore, a number of measures are frequently cited to guarantee safety and efficacy, including frequent monitoring of raw materials and completed products, quantitative analysis of these compounds to ensure consistent therapeutic effects, thorough testing for potential contaminants, application of GMP principles in the production process, and post-market surveillance. Continuous quality control procedures allow for the quick identification and resolution of any problems pertaining to the safety or quality of the product (Wang *et al.,* 2023). The authenticity of the herbs used in the products can be confirmed through the use of advanced methods like DNA barcoding. Strong quality control procedures guarantee the efficacy and integrity of herbal medicines, building consumer confidence and encouraging the ethical use of herbal medicine into contemporary medical procedures.

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References

Abd Kadir, S. L., Yaakob, H. and Mohamed Zulkifli, R. (2013). Potential anti-dengue medicinal plants: a review. Journal of natural medicines, 67:677-689.

Ahmad, N., Fazal, H., Ayaz, M., Abbasi, B. H., Mohammad, I. and Fazal, L. (2011). Dengue fever treatment with Carica papaya leaves extracts. Asian Pacific journal of tropical biomedicine, 1:330-333.

An, J., Kimura-Kuroda, J., Hirabayashi, Y. and Yasui, K. (1999). Development of a novel mouse model for dengue virus infection. Virology, 263:70-77.

Arawwawala, M., Thabrew, I., Arambewela, L. and Handunnetti, S. (2010). Anti-inflammatory activity of Trichosanthes cucumerina Linn. in rats. Journal of ethnopharmacology, 131:538-543.

Azeredo, E. L. D., Monteiro, R. Q. and de-Oliveira Pinto, L. M. (2015). Thrombocytopenia in dengue: interrelationship between virus and the imbalance between coagulation and fibrinolysis and inflammatory mediators. Mediators of inflammation, 2015.

Babu, N., Srivastava, S. K., Prusty, M. and Sahoo, T. (2016). Medicinal and aromatic plant production technologies: A step towards farm women prosperity.

Barde, P. V., Mishra, N. and Singh, N. (2018). Timely diagnosis, use of information technology and mosquito control prevents dengue outbreaks: Experience from central India. Journal of infection and public health, 11:739-741.

Basu, A., Jain, P., Gangodkar, S. V., Shetty, S. and Ghosh, K. (2008). Dengue 2 virus inhibits in vitro megakaryocytic colony formation and induces apoptosis in thrombopoietin-inducible megakaryocytic differentiation from cord blood CD34+ cells. FEMS Immunology & Medical Microbiology, 53:46-51.

Benencia, F. and Courreges, M. C. (1999). Antiviral activity of sandalwood oil against herpes simplex viruses-1 and-2. Phytomedicine, 6:119-123.

Biswal, S., Borja-Tabora, C., Vargas, L. M., Velásquez, H., Alera, M. T., Sierra, V. and Tricou, V. (2020). Efficacy of a tetravalent dengue vaccine in healthy children aged 4–16 years: a randomised, placebo-controlled, phase 3 trial. The Lancet, 395:1423-1433.

Biswal, S., Reynales, H., Saez-Llorens, X., Lopez, P., Borja-Tabora, C., Kosalaraksa, P., ... and Wallace, D. (2019). Efficacy of a tetravalent dengue vaccine in healthy children and adolescents. New England Journal of Medicine, 381:2009-2019.

Bordia, A., Verma, S. K. and Srivastava, K. C. (1997). Effect of ginger (*Zingiber officinale* Rosc.) and fenugreek (*Trigonella foenumgraecum* L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins, leukotrienes and essential fatty acids, 56:379-384.

Bulich, R. and Aaskov, J. G. (1992). Nuclear localization of dengue 2 virus core protein detected with monoclonal antibodies. Journal of General Virology, 73:2999-3003.

Chan, J. F. W., Chik, K. K. H., Yuan, S., Yip, C. C. Y., Zhu, Z., Tee, K. M., ... and Yuen, K. Y. (2017). Novel antiviral activity and mechanism of bromocriptine as a Zika virus NS2B-NS3 protease inhibitor. Antiviral research, 141:29-37.

Chiang, C. Y., Pan, C. H., Chen, M. Y., Hsieh, C. H., Tsai, J. P., Liu, H. H. and Chen, H. W. (2016). Immunogenicity of a novel tetravalent vaccine formulation with four recombinant lipidated dengue envelope protein domain IIIs in mice. Scientific reports, 6:30648.

Deen, J. (2016). The dengue vaccine dilemma: balancing the individual and population risks and benefits. PLoS medicine, 13:e1002182.

Dejnirattisai, W., Jumnainsong, A., Onsirisakul, N., Fitton, P., Vasanawathana, S., Limpitikul, W. and Screaton, G. (2010). Cross-reacting antibodies enhance dengue virus infection in humans. Science, 328:745-748.

Dharmarathna, S. L. C. A., Wickramasinghe, S., Waduge, R. N., Rajapakse, R. P. V. J., and Kularatne, S. A. M. (2013). Does Carica papaya leaf-extract increase the platelet count? An experimental study in a murine model. Asian Pacific Journal of Tropical Biomedicine, 3:720-724.

Dn, F. and Rt, C. (2014). Review of current dengue treatment and therapeutics in development. Journal of Bioanalysis & Biomedicine, 8.

Ferreira-de-Lima, V. H. and Lima-Camara, T. N. (2018). Natural vertical transmission of dengue virus in Aedes aegypti and Aedes albopictus: a systematic review. Parasites & vectors, 11:1-8.

Gowda, A. C., Kumar, N. V., Kasture, P. N. and Nagabhushan, K. H. (2015). A Pilot Study to Evaluate the Effectiveness of Carica Papaya Leaf Extract in Increasing the Platelet Count in Cases of Dengue with Thrombocytopenia.

Guzman, M. G., Hermida, L., Bernardo, L., Ramirez, R. and Guillén, G. (2010). Domain III of the envelope protein as a dengue vaccine target. Expert review of vaccines, 9:137-147.

Hadinegoro, S. R., Arredondo-García, J. L., Capeding, M. R., Deseda, C., Chotpitayasunondh, T., Dietze, R. and Saville, M. (2015). Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. New England Journal of Medicine, 373:1195-1206.

Halstead, S. B., Mahalingam, S., Marovich, M. A., Ubol, S. and Mosser, D. M. (2010). Intrinsic antibody-dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes. The Lancet infectious diseases, 10:712-722. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (2024)

Huang, J., Li, Y., Qi, Y., Zhang, Y., Zhang, L., Wang, Z. and Gui, L. (2014). Coordinated regulation of autophagy and apoptosis determines endothelial cell fate during Dengue virus type 2 infection. Molecular and cellular biochemistry, 397:157-165.

Iglesias, N. G., Mondotte, J. A., Byk, L. A., De Maio, F. A., Samsa, M. M., Alvarez, C. and Gamarnik, A. V. (2015). Dengue virus uses a non-canonical function of the host GBF1-Arf-COPI system for capsid protein accumulation on lipid droplets. Traffic, 16:962-977.

Jin, X., Block, O. T., Rose, R. and Schlesinger, J. (2009). Dengue vaccine development and dengue viral neutralization and enhancement assays. Antiviral therapy, 14:739-749.

Johnson, A. J. and Roehrig, J. T. (1999). New mouse model for dengue virus vaccine testing. Journal of virology, 73:783-786.

Khetarpal, N. and Khanna, I. (2016). Dengue fever: causes, complications, and vaccine strategies. Journal of immunology research, 2016.

Kilani, S., Ammar, R. B., Bouhlel, I., Abdelwahed, A., Hayder, N., Mahmoud, A. and Chekir-Ghedira, L. (2005). Investigation of extracts from (Tunisian) Cyperus rotundus as antimutagens and radical scavengers. Environmental Toxicology and Pharmacology, 20:478-484.

Kim, H. J., Chen, F., Wang, X., Chung, H. Y. and Jin, Z. (2005). Evaluation of antioxidant activity of vetiver (*Vetiveria zizanioides* L.) oil and identification of its antioxidant constituents. Journal of agricultural and food chemistry, 53:7691-7695.

Kocienski, P. (2021). Synthesis of Dengue Virus Inhibitor JNJ-A07. Synfacts, 18:0006.

Koishi, A. C., Zanello, P. R., Bianco, É. M., Bordignon, J. and Nunes Duarte dos Santos, C. (2012). Screening of Dengue virus antiviral activity of marine seaweeds by an in-situ enzyme-linked immunosorbent assay. PLoS One, 7:e51089.

Kudineer, N. (2013). A combination of Nilavembu Kudineer and Adathodai Manapagu in the management of dengue fever.

Lazo Vázquez, L., Gil González, L., Marcos López, E., Pérez Fuentes, Y., Cervetto de Armas, L., Brown Richards, E. and Hermida Cruz, L. (2017). Evaluation in mice of the immunogenicity of a tetravalent subunit vaccine candidate against dengue virus using mucosal and parenteral immunization routes. Viral Immunology, 30:350-358.

Lee, S. H., Tang, Y. Q., Rathkrishnan, A., Wang, S. M., Ong, K. C., Manikam, R. and Sekaran, S. D. (2013). Effects of cocktail of four local Malaysian medicinal plants (*Phyllanthus* spp.) against dengue virus 2. BMC complementary and alternative medicine, 13:1-13.

Li, Q. and Kang, C. (2022). Structures and dynamics of dengue virus nonstructural membrane proteins. Membranes, 12:231.

Lin, L., Koren, M. A., Paolino, K. M., Eckels, K. H., De La Barrera, R., Friberg, H. and Thomas, S. J. (2021). Immunogenicity of a live-attenuated dengue vaccine using a heterologous prime-boost strategy in a phase 1 randomized clinical trial. The Journal of Infectious Diseases, 223:1707-1716.

Lin, Y. L., Liao, C. L., Chen, L. K., Yeh, C. T., Liu, C. I., Ma, S. H. and King, C. C. (1998). Study of Dengue virus infection in SCID mice engrafted with human K562 cells. Journal of virology, 72:9729-9737.

Lin, Y. P., Luo, Y., Chen, Y., Lamers, M. M., Zhou, Q., Yang, X. H. and Liu, Z. M. (2016). Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. BMC infectious diseases, 16:1-8.

Liu, Y., Liu, J. and Cheng, G. (2016). Vaccines and immunization strategies for dengue prevention. Emerging microbes & infections, 5:1-6.
Low, J. G., Ooi, E. E. and Vasudevan, S. G. (2017). Current status of dengue therapeutics research and development. The Journal of infectious diseases, 215:S96-S102.
Macaulay, I. C., Tijssen, M. R., Thijssen-Timmer, D. C., Gusnanto, A., Steward, M., Burns, P. and Ouwehand, W. H. (2007). Comparative gene expression profiling of in vitro differentiated megakaryocytes and erythroblasts identifies novel activatory and inhibitory platelet membrane proteins. Blood, 109:3260-3269.
Manoff, S. B., Sausser, M., Russell, A. F., Martin, J., Radley, D., Hyatt, D. and Coller, B. A. (2019). Immunogenicity and safety of an investigational tetravalent recombinant subunit vaccine for dengue: Results of a Phase I randomized clinical trial in flavivirus-naïve adults. Human vaccines & immunotherapeutics.
Meghwal, M. and Goswami, T. K. (2013). Piper nigrum and piperine: an update. Phytotherapy Research, 27:1121-1130.
Moi, M. L., Lim, C. K., Chua, K. B., Takasaki, T. and Kurane, I. (2012). Dengue virus infection-enhancing activity in serum samples with neutralizing activity as determined by using FcγR-expressing cells. PLoS neglected tropical diseases, 6:e1536.
Moquin, S. A., Simon, O., Karuna, R., Lakshminarayana, S. B., Yokokawa, F., Wang, F. and Gu, F. (2021). NITD-688, a pan-serotype inhibitor of the dengue virus NS4B protein, shows favorable pharmacokinetics and efficacy in preclinical animal models. Science Translational Medicine, 13:eabb2181.
Murphy, B. R. and Whitehead, S. S. (2011). Immune response to dengue virus and prospects for a vaccine. Annual review of immunology, 29:587-619.
Padilla-s, L., Rodríguez, A., Gonzales, M. M., Gallego-g, J. C. and Castaño-o, J. C. (2014). Inhibitory effects of curcumin on dengue virus type 2-infected cells in vitro. Archives of virology, 159:573-579.
Pangtey, G. S., Prakash, A. and Munjal, Y. P. (2016). Role of Carica papaya leaf extract for dengue associated thrombocytopenia. Journal of the Association of Physicians of India, 64:11-13.
Park, J., Kim, J. and Jang, Y. S. (2022). Current status and perspectives on vaccine development against dengue virus infection. Journal of Microbiology, 60:247-254.
Paschapur, M. S., Patil, M. B., Kumar, R. and Patil, S. R. (2009). Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals. Journal of Medicinal Plants Research, 3:049-054.
Perng, G. C., Lei, H. Y., Lin, Y. S. and Chokephaibulkit, K. (2011). Dengue vaccines: challenge and confrontation.
Porter, K. R., Ewing, D., Chen, L., Wu, S. J., Hayes, C. G., Ferrari, M. and Raviprakash, K. (2012). Immunogenicity and protective efficacy of a vaxfectin-adjuvanted tetravalent dengue DNA vaccine. Vaccine, 30:336-341.
Putintseva, E., Vega, G. and Fernandez, L. (1986). Alterations in thrombopoiesis in patients with thrombocytopenia produced by dengue hemorrhagic fever. Nouvelle revue francaise d'hematologie, 28:269-273.
Ranasinghe, P., Ranasinghe, P., Abeysekera, W. K. M., Premakumara, G. S., Perera, Y. S., Gurugama, P. and Gunatilake, S. B. (2012). In vitro erythrocyte membrane stabilization properties of Carica papaya L. leaf extracts. Pharmacognosy research, 4:196.
Rosa, B. R., da Cunha, A. J. L. A. and de Andrade Medronho, R. (2019). Efficacy, immunogenicity and safety of a recombinant tetravalent dengue vaccine (CYD-TDV) in children aged 2–17 years: systematic review and meta-analysis. BMJ open, 9.
Roth, C., Cantaert, T., Colas, C., Prot, M., Casadémont, I., Levillayer, L. and Sakuntabhai, A. (2019). A modified mRNA vaccine targeting immunodominant NS epitopes protects

against dengue virus infection in HLA class I transgenic mice. Frontiers in Immunology, 10:451080.

Sadique, J., Chandra, T., Thenmozhi, V. and Elango, V. (1987). The anti-inflammatory activity of Enicostemma littorale and Mollugo cerviana. Biochemical medicine and metabolic biology, 37:167-176.

Sangiambut, S., Keelapang, P., Aaskov, J., Puttikhunt, C., Kasinrerk, W., Malasit, P. and Sittisombut, N. (2008). Multiple regions in dengue virus capsid protein contribute to nuclear localization during virus infection. Journal of General Virology, 89:1254-1264.

Slifka, M. K. and Amanna, I. J. (2018). Dengue Serostatus and Dengue Vaccine Safety and Efficacy. The New England journal of medicine, 379:1968-1968.

Sridhar, S., Luedtke, A., Langevin, E., Zhu, M., Bonaparte, M., Machabert, T. and DiazGranados, C. A. (2018). Effect of dengue serostatus on dengue vaccine safety and efficacy. New England Journal of Medicine, 379:327-340.

Sun, W., Cunningham, D., Wasserman, S. S., Perry, J., Putnak, J. R., Eckels, K. H. and Edelman, R. (2009). Phase 2 clinical trial of three formulations of tetravalent live-attenuated dengue vaccine in flavivirus-naive adults. Human vaccines, 5:33-40.

Tang, L. I., Ling, A. P., Koh, R. Y., Chye, S. M. and Voon, K. G. (2012). Screening of anti-dengue activity in methanolic extracts of medicinal plants. BMC complementary and alternative medicine, 12:1-10.

Tsai, W. Y., Lin, H. E. and Wang, W. K. (2017). Complexity of human antibody response to dengue virus: implication for vaccine development. Frontiers in microbiology, 8:1372.

Vannice, K. S., Durbin, A. and Hombach, J. (2016). Status of vaccine research and development of vaccines for dengue. Vaccine, 34:2934-2938.

Vasudevan, P., Kashyap, S. and Sharma, S. (1999). Bioactive botanicals from basil (*Ocimum* sp.). Journal of Scientific & Industrial Research, https://nopr.niscair.res.in/bitstream/123456789/17820/1/JSIR%2058(5)%20332-338.pdf

Wang, H., Chen, Y. and Wang, C. (2023). Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Frontiers in Pharmacology, 14:1265178.

Wang, Q. Y., Kondreddi, R. R., Xie, X., Rao, R., Nilar, S., Xu, H. Y. and Shi, P. Y. (2011). A translation inhibitor that suppresses dengue virus in vitro and in vivo. Antimicrobial agents and chemotherapy, 55:4072-4080.

Wang, W. K., Sung, T. L., Lee, C. N., Lin, T. Y. and King, C. C. (2002). Sequence diversity of the capsid gene and the nonstructural gene NS2B of dengue-3 virus in vivo. Virology, 303:181-191.

Whitehead, S. S., Durbin, A. P., Pierce, K. K., Elwood, D., McElvany, B. D., Fraser, E. A. and Kirkpatrick, B. D. (2017). In a randomized trial, the live attenuated tetravalent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination. PLoS neglected tropical diseases, 11:e0005584.

Whitehorn, J. and Simmons, C. P. (2011). The pathogenesis of dengue. Vaccine, 29:7221-7228.

Wollner, C. J. and Richner, J. M. (2021). mRNA vaccines against flaviviruses. Vaccines, 9:148.

Wollner, C. J., Richner, M., Hassert, M. A., Pinto, A. K., Brien, J. D. and Richner, J. M. (2021). A dengue virus serotype 1 mRNA-LNP vaccine elicits protective immune responses. Journal of Virology, 95:10-1128.

Xu, J., Xie, X., Chen, H., Zou, J., Xue, Y., Ye, N. and Zhou, J. (2020). Design, synthesis and biological evaluation of spiropyrazolopyridone derivatives as potent dengue virus inhibitors. Bioorganic & medicinal chemistry letters, 30:127162.

Zellweger, R. M. and Shresta, S. (2014). Mouse models to study dengue virus immunology and pathogenesis. Frontiers in immunology, 5:151.

Zhang, J. S., Zhao, N. N., Liu, Q. Z., Liu, Z. L., Du, S. S., Zhou, L. and Deng, Z. W. (2011). Repellent constituents of essential oil of Cymbopogon distans aerial parts against two stored-product insects. Journal of Agricultural and Food Chemistry, 59:9910-9915.

Zhang, M., Sun, J., Li, M. and Jin, X. (2020). Modified mRNA-LNP Vaccines confer protection against experimental DENV-2 infection in mice. Molecular Therapy – Methods & Clinical Development, 18:702-712.

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