Molecular herbal inhibitors of Dengue virus: an update

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Abstract: Dengue fever is the most prevalent mosquito transmitted viral infection affecting 2.5 billion people across the globe. Etiological agent (dengue virus) is an enveloped positive sense RNA virus belonging to the family Flaviviridae. It has four serotypes which does not provide cross protection immunity against each other making vaccine development difficult due to antibody-dependent enhancement effect. Till now there is no approved vaccine or drug against this virus. Therefore, there is an urgent need of development of alternative solutions for dengue. In traditional medicine system plants extracts have been used to cure several human ailments. Several plant species have been reported with anti-dengue activity. However, mechanism of action of only some of the anti-dengue herbal compounds isolated from medicinal plants have been elucidate while other needs further investigations. With the advent of synthetic biology and metagenomics and high throughput screening, it have become possible to screen and produce plant based effector molecules into microorganism like E. coli at larger amount and at relatively low cost. Networking is the key of success. Networking among pure scientist, clinicians, applied scientist and industry people can only make possible to available these natural bioactive compounds against dengue virus from nature to bedside for humankind.

Keywords: Dengue; medicinal plants; patents; inhibitors; anti-dengue herbal drugs; scientific networking.

Introduction

Dengue is the most prevalent mosquito borne viral infection in the world (WHO 2009; Guzman et al. 2010; Whitethorn and Farrar 2010). The name Dengue virus (DENV) refers to a group of four antigenically and genetically related viruses called serotypes (DENV1-4) (Halstead 1988; WHO 2009; Whitethorn and Farrar 2010; Chen et al. 2011; Rothman 2011). Each serotype is capable of causing dengue fever and manifests in severe forms (dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS)) (Gubler 1998; WHO 2009; Whitethorn and Farrar 2010; Murphy and Whitehead 2011). According to the World Health Organization (WHO), nearly 2.5 billion people are at the risk...
of dengue virus infection (WHO 2009) (Figure 1). Dengue is endemic in more than 100 countries in Asia, Pacific, America, Africa, and Caribbean (Figure 1). Dengue manifests in both epidemics as well as sporadic forms (Gubler 1998; WHO 2009; Thomas and Endy 2011). During epidemics of dengue, infection rates among those who have not been previously exposed to the virus are often 40% to 50%, but can reach up to 80% to 90% (Gibbons et al. 2007; Kyle and Harris 2008; WHO 2009). An estimated 500,000 people with DHF require hospitalization each year, a significant proportion of whom are children with mortality rate 2.5% among infected people (Singhi et al. 2007; WHO 2009; Thomas and Endy 2011).

Several approaches have been employed to fight with DENV including monoclonal antibodies (Sukupolvi-Petty et al. 2010; Deng et al. 2011; Cockburn et al. 2012), RNAi technology (Sanchez-Vargas et al. 2004; Stein and Shi 2008; Wu et al. 2010; Lee et al. 2010; Subramanya et al. 2010; Alhoot et al. 2011; Burnett et al. 2011; Stein et al. 2011; Aliabadi et al. 2012; Foged 2012;) vaccines (Durbin and Whitehead 2011) which include live attenuated vaccine (Guirakho et al. 2006; Kitchener et al. 2006; Guy and Almond 2008; Simmons et al. 2010); subunit tetravalent vaccine (Batra et al. 2010; Morrison et al. 2010; Watanaveeradej et al. 2011), inactivated vaccines (Maves et al. 2010, 2011; Simmons et al. 2010); DNA vaccines (Azevedo et al. 2011; Costa et al. 2011; Danko et al. 2011). However, till now there is no licensed vaccine or drug available in the market for dengue. Prevention measures are insufficient and only supportive therapy is available (Clarke 2002; WHO 2009; Thomas and Endy 2011; Whitehorn and Simmons 2011).

Hence, there is an urgent need to find alternate solutions to combat dengue. Plants and plant derived compounds remain an important source for the discovery and the development of new antiviral drugs because of their expected low side effects and their high accessibility in the nature (Balandrin et al. 1985; Manuel et al. 1993; Rates 2001; Dewick 2002; Schmidt et al. 2007; Saklani and Kutty 2008; Adams et al. 2011; Bruno 2011; Cordell 2011). This review will provide an update on our understanding of pathogenesis of this virus and summarize success stories of medicinal plants against DENV and how far are they from the target?
**Dengue transmission**

The mosquitoes *Aedes aegypti* and *Aedes albopictus*, are main culprits responsible for transmitting dengue between people and are cosmopolitan in nature (Kyle and Harris 2008; WHO 2009; Guzman and Istúriz 2010; Lambrechts et al. 2010; Ross 2010; Urdaneta-Marquez and Failloux 2011). The life cycle of dengue virus involves two hosts: mosquito as vector, and targets humans.

When an infected mosquito bites a person, it injects the DENV into the bloodstream of the host. Subsequently virus infects nearby skin cells called keratinocytes, the most common cell type in the skin. In addition dengue virus also infects and replicates inside a specialized immune cell located in the skin, a type of dendritic cell called a Langerhans cell (NATURE EDUCATION). Once infected, humans become a reservoir and source of more viruses generated during viral replication in host and thus serve as a source of the virus for uninfected mosquitoes (Adams and Kapan 2009; WHO 2009; Omenn 2010). Patients who are already infected with the dengue virus can transmit the infection via *Aedes* mosquitoes. Subsequently, mosquito mainly acquires the virus while feeding on the blood of an infected person (WHO 2009; Guzman et al. 2010; Whitethorn and Farrar 2010).

Within the mosquito, DENV travels to the mid-gut of mosquito and afterward spreads to the salivary glands in a time period of 8-12 days (WHO 2009). After this incubation period (in mosquito), the virus is ready for being transmitted to humans during probing or feeding.

It is worth mentioning here that *Aedes* mosquito is a holometabolous insect which means that the mosquito goes through a complete metamorphosis with an egg, larvae, pupae, and finally adult stage (Urdaneta-Marquez and Failloux 2011) (Figure 2). Average life span ranges from 14 to 30 days based on environmental conditions. After taking a human blood meal (Hansen et al. 2004; Bryant et al. 2010) female *Aedes aegypti* mosquito can produce on average 100 to 200 eggs per batch (Wong et al. 2011). The females can produce up to five batches of eggs during a lifetime which means 500-1000 (Wong et al. 2011) eggs in a lifetime (Figure 2).

This gives a fair idea of chances of getting of dengue infection in an endemic region.

**Figure 2**: Life cycle of vector *Aedes aegypti* responsible for transmission of dengue. (From http://www.denguevirusnet.com/life-cycle-of-aedes-aegypti.html)

**Symptoms**

Primary infection (for the first time) with dengue virus is usually asymptomatic or with mild symptoms which include high fever, retro-orbital pain (pain behind eyes), low platelet count, joint and muscles pain which are non specific (WHO 2009; Guzman et al. 2010; Whitehorn and Farrar 2010; Whitehorn and Simmons 2011). Secondary infection with another DENV serotype can prove lethal with high morbidity and mortality and manifests in the form of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler 1998; WHO 2009; Faheem et al. 2011). Symptoms usually appear in 3-14 days (average 4–7 days) after the infective female *Aedes* bite.

Dengue hemorrhagic fever (DHF) is characterized by increased vascular permeability, hypovolaemia (low blood volume), and abnormal blood clotting mechanisms. DHF is a potentially deadly complication with symptoms similar to those of dengue fever (Gubler 1998; Potts and Rothman 2008; WHO 2009; Pawitan 2011; Guzman et al. 2010; Guzman and Vazquez 2010; Rodenhuis-Zybert et al. 2010; Srikiatkhachorn and Green 2010; Ranjit and Kissoon 2011).

The dengue Shock Syndrome (DSS) is characterized by bleeding that may appear as tiny spots of blood on the skin (petechiae) and larger patches of blood under the skin (ecchymoses).
Shock may result in death within 12 to 24 hours. Patients can recover following timely supportive medical treatments (Kabra et al. 1999; WHO 2009).

Our immunity and Dengue virus

Our adaptive immune response to DENV infection contributes to resolution of the infection (Murphy and Whitehead 2011). Dengue antibodies against one serotype can prevent the infection of those specific serotypes and provide lifelong immunity against that particular serotype. That’s why primary Dengue infection is usually asymptomatic. However, this temporary immunity usually wanes after 6 months, the point at which an individual is susceptible to the other three DENV serotypes (Sabin 1952; Balakrishnan et al. 2011; Dowd and Pierson 2011). Infection with a different serotype leads to secondary infection as well as antibody dependent enhancement (ADE) (Dowd and Pierson 2011; Murphy and Whitehead 2011).

Diagnosis of Dengue

Diagnosis of dengue is based on serology (ELISA) detection of IgM, IgG in patients (Gowri Sankar et al. 2012). Detection of dengue virus nucleic acid (RNA) in human blood is performed by Reverse transcriptase PCR. Leucopenia (decrease in leukocytes counts), thrombocytopenia (decrease in platelet count) are also good indicators of dengue fever but are nonspecific. However, isolation of dengue virus is the most reliable way to diagnose the dengue. But facility to isolate is not frequently available especially in developing countries (Peeling et al. 2010; Peh et al. 2011).

Dengue virus: Molecular organization

Dengue virus (DENV) belongs to the family Flaviviridae, genus Flavivirus (ICTV 2005), is a small (~50 nm), enveloped, single-stranded, positive sense RNA virus (~11 kb in size) with icosahedral symmetry (Figure 3A).

The 5’ end of the virus is protected by a class II G cap followed by a short untranslated region (~100 nt) (NCR) which plays a critical role in viral replication as well as translation (Iglesias and Gamarnik 2011). The 3’ NCR of the virus, which is relatively longer than the 5’ end (~450 bp) has several conserved RNA structures but lacks a terminal poly A tail (Iglesias and Gamarnik 2011) (Figure 3A). These conserved structures are necessary for DENV replication and also regulates translation.

Figure 3A: Schematic diagram of dengue virus genome and polyprotein encoded by single open reading frame (From Tomlinson et al., 2009) (for description see text).

Figure 3B: Outline of the dengue virus replication cycle (for description see text) (From Tomlinson et al. 2009)

Replication cycle Dengue Virus in Human: unique protein- potent drug

Briefly, the first step of replication is the attachment of virus to the specific receptor present on the host cells (Tomlinson et al. 2009; Smit et al. 2011; Smith 2012). The envelope (E) glycoprotein present on DENV membrane acts
as ligand that binds to receptors present on the host cell membrane. It initiates the interaction between E-glycoprotein and the host receptor(s) which leads to receptor mediated endocytosis of the virus particle (Tomlinson et al. 2009; Smith et al. 2011) (Figure 3B). Further acidification of the endocytic vesicle; release the entrapped nucleocapsid to the cytoplasm where the virus genome is released. DENV is a positive sense RNA virus with single open reading frame (ORF) thus behave like messenger RNA and directly translates to produce a large single polyprotein which subsequently undergoes post translational modification mediated by viral as well as host proteases to produce seven non structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) which are necessary for virus replication and three structural proteins (capsid, pre membrane and envelope) which are involved in packaging and secretion of the DENV from infected cells (Tomlinson et al. 2009) (Table 1). Replication occurs on intercellular membranes, and assembly take place on the endoplasmic reticulum. Newly assembled virion are transported through trans-golgi network and released from cell by exocytosis (Mukhopadhyay et al. 2005; Tomlinson et al. 2009) (Figure 3B).

**Table 1:** Summarizing putative and characterized function of proteins encoded by dengue virus.

<table>
<thead>
<tr>
<th>Protein functional class</th>
<th>Protein Name</th>
<th>Function</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Non Structural Proteins</td>
<td>Non Structural (NS) protein 1</td>
<td>Essential for virus growth. May play role in pathogenesis of Dengue by contributing to endothelium dysfunction and also has immune evasive functions. However, precise function is not known.</td>
<td>Avirutnan et al. (2006), Gutsche et al. (2011), Somnueke et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>NS2A</td>
<td>Little information is available. NS2A has been associated with down-regulation of IFN-β-stimulated gene expression</td>
<td>Muñoz-Jordan et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>NS2B</td>
<td>Act as a cofactor for viral protease (NS3)</td>
<td>Chambers et al. (1990), Zuo et al. (2009), Phong et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>NS3</td>
<td>Multifunctional protein. Possess NTPase, helicase, and RTase activities which help in unwinding of double stranded RNA formed during replication.</td>
<td>Bartelma and Padmanabhan (2002), Zuo et al. (2009), Su et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>NS4A</td>
<td>Precise function not known. However, it is critical determinant of Dengue replication.</td>
<td>McLean et al. (2011), Muñoz-Jordan et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>NS4B</td>
<td>Function is poorly understood. However, role in innate immune signaling and virulence is postulated.</td>
<td>Grant et al. (2011), Kelley et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>NS5</td>
<td>Multifunctional protein having Polymerase (RdRP), Methyltransferase activity. Beside recently NS5 has been shown to bind STAT2 and inhibits IFN dependent signaling.</td>
<td>Ackermann and Padmanabhan (2001), Egloff et al. (2002), Ashour et al. (2009), Dong et al. (2010), Iglesias et al. (2011)</td>
</tr>
<tr>
<td>Structural Proteins</td>
<td>Capsid</td>
<td>Necesssary for packaging and release of viral particles from the cell</td>
<td>Nowak et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>prM protein</td>
<td>Protects the envelope protein from premature fusion during transit through the acidic environment of the trans-golgi network.</td>
<td>Nowak et al. (1989), Crill and Roehrig (2001), Lin et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Envelope</td>
<td>Fusion and entry of virus into host cell and cell attachment</td>
<td>Nowak et al. (1989), Crill and Roehrig (2001), Lin et al. (2011)</td>
</tr>
</tbody>
</table>

The detailed function of dengue proteins involved in the viral replication is largely unknown. The role of NS1 is largely unknown but is essential for virus survival (Table 1). It is a soluble glycoprotein detected very early during infection thus considered to be a good diagnostic marker for Dengue. NS2A, NS2B, NS4A, and NS4B are membrane –associated proteins believed to anchor and regulate the replication complex during the viral life-cycle (Table 1). NS2B-NS3 is a serine protease necessary for the processing of polyprotein critical for virus replication (Bollati et al. 2010; Keller et al. 2006). NS5 is a multifunctional, the most high-
ly conserved of the dengue proteins protein and possesses RNA Dependent RNA polymerase activity, key and unique to the virus as RdRp is absent in mammals (Keller et al. 2006; Rawlinson et al. 2006; Malet et al. 2008; Alcaraz-Estrada et al. 2010; Bollati et al. 2010). NS5 RdRp domain is responsible for the generation of negative sense RNA which further act as template for the production of positive sense RNA for packaging. Viral proteins involved in replication cycle of the virus have been used as targets for the development of new antiviral agents.

Alternative approach: Medicinal Plants: can they combat DENV?

There is a long history of use of Plants to cure human diseases throughout the world (van Agtmael et al. 1999; Li and Vederas 2009). Several plant derivatives have been successfully entered clinical trials (Butler 2004; Koehn and Carter 2005; Harvey 2008; Saklani and Kutty 2008). It is noteworthy that few studies have shown that extract from different parts of plants provide better antiviral results compared to their synthetic analogues (Chiang et al. 2005; Bruno 2011; Tang et al. 2012). As such, the development of a plant based antiviral preparation promises a more potential alternative in combating dengue disease. Especially the World Health Organization (WHO) advocated that developing and developed countries should collaborate with traditional medicine practitioners to explore new aspects that provide safe, economical, and effective remedies for viral diseases (WHO 2009; Bruno 2011; Tang et al. 2012).

According to WHO in some Asian and African countries, nearly 80% of the population depend on traditional medicine for primary health care (Grayson 2011). Out of which the herbal medicines are on the top of list which also strengthen the economy of several countries and are quite popular in the international market. Herbal medicines refer to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients. However, it is noteworthy that herbal medicine are not risk free and sometime can be lead to adverse consequences if preparation is of poor quality (Gilbert 2011; Grayson 2011; Sakurai 2011). Strict regulation should be pose which can help in developing safe and cheap herbal medicine. Unlike pharmaceuticals drugs for which detailed pharmacokinetics and molecular mechanism of inhibitor is known, herbal medicines mostly lack such data (Tu 2011; Xu et al. 2012).

In further in this review, we have grouped medicinal plants on the basis of their inhibitory effect on replication cycle of dengue virus which will help the reader to understand their molecular basis. However, several relevant studies are present where anti-Dengue activity has been demonstrated by plant extract by several groups. But their systemic pharmacological examination and molecular basis have not yet been well characterized and needs further investigations.

Medicinal Plants: Virus Entry Inhibitor

As described earlier, first step of viral infectious replication cycle is the attachment to the susceptible host via receptor mediated interactions followed by entry into host cell (Figure 3B) (Hase et al. 1989). Inhibiting virus host interaction at this step would be the most efficient strategy against any viral infection. In dengue Envelope protein Domain III is the receptor-binding motif thus critical for virus infection as it mediate host virus interaction (Crill and Roehrig, 2001).

Tong et al. (2010) in their study have demonstrated the inhibitory potential of the compound WSS45 (sulfate derivative of an alpha-D-glucan) derived from Gastrodia elata Bl. (a saprophytic perennial herb which is widely used in traditional Chinese medicine preparation) has documented antiviral potential against dengue virus serotype 2 in vitro using BHK cell line (Baby hamster kidney fibroblast cells). Using virus adsorption and virus cell penetration assay they clearly demonstrate that WSS45 interfere with adsorption of the DENV to the host cell without significant cytotoxicity (Tong et al. 2010). However, study was purely in vitro in nature and restricted to DENV-2 only. Furthermore, the ineffectiveness of WSS45 after virus

http://www.openaccessscience.com
jijmap@openaccessscience.com
enters the susceptible cell strongly suggests that it is a viral entry inhibitor (Tong et al. 2010).

**Carrageenans: as virus entry inhibitor**

The term Carrageenans refers to the family of natural polysaccharides extracted from red sea weeds (Irish moss, *Chondrus crispus*). Chemically they are linear sulfate polysaccharides. There are several varieties of carrageenans (kappa, lambda, Iota) which have been reported with potent antiviral effect e.g. papillomavirus virus (Buck et al. 2006; Roberts et al. 2007). Recently clinical trial of iota carrageenans based nasal spray has been evaluated against common cold (Eccles et al. 2010).

In another significant study, Talarico and Damonte (2007) investigated the effect of lambda and iota carrageenans sulfate polysaccharides containing linear chains of galactopyranosyl residues on multiplication of DENV-2 and DENV-3 in Vero (African green monkey Kidney epithelium cells) and HepG2 cells (human liver hepatocellular carcinoma cell line). Their revealed that tested carrageenans were potent inhibitors of DENV-2 in. Virus growth inhibition was demonstrated by plaque reduction, virus yield inhibition and antigen expression assays. Further using virus internalization assay and real time PCR they demonstrated that lambda-carrageenans hinder with the virus adsorption to the host cell as well in internalization step (Talarico and Damonte 2007). It is noteworthy that lambda- carrageenans are heparan sulfate imitative compound. Heparan sulfate has been known as receptor or co receptor for several viruses (Spillmann 2001; Liu and Thorp 2002). In addition using infectious center and virion uncoating assays, they demonstrate that carrageenan-treated virions remain trapped within endosomes (Talarico and Damonte 2007). Furthermore, no inhibition in virus growth was observed when entry step was eliminate using DENV 2 RNA transfection strongly indicates that lambda-carrageenan is an effective plant derived dengue virus entry inhibitor.

**NS2B-NS3 inhibitors**

NS2B acts as a cofactor for the NS2B-NS3 active serine protease. The protease domain of NS3 needs the presence of 40 aa of NS2B to form an active protease site. Polyprotein processing is an important and the foremost step in the replication cycle of DENV (Figure 3B). Even chances of defective virus are not much as DENV has single ORF. That is the reason why NS2b/NS3 protease has been the first dengue protein target actively used in drug design programs (Chambers et al, 1990; Bartelma and Padmanabhan 2002; Su et al. 2009; WHO 2009; Tomlinson et al. 2009; Zuo et al. 2009; Phong et al. 2011; Yang et al. 2011).

Kiat et al (2006) studied the effect of groups of falvanones and their chalcones against protease of DENV-2. Source of these bioactive compounds was *Boesenbergia rotunda* (L.), commonly known as Chinese ginger or Fingeringoot. Their results indicate that cyclohexenyl chalcone derivatives (4-hydroxypanduratin A and panduratin A) were potent competitive inhibitors of DENV-2 NS3 protease in vitro. Whereas pinostrobin and cardamonin were observed to be non-competitive inhibitor (Kiat et al. 2006). Same group further support their data by automated docking studies which is in arrangement with their experimental data (Othman et al. 2008).

**Cyclotides and Dengue**

Cyclotides are low molecular weight (28-47 aa) plant defense proteins (Craik et al. 2004, 2010; Mulvenna et al. 2004). Beside insecticidal properties (Barbeta et al. 2008) these mini-proteins have wide range of other pharmaceutical activities (Craik et al. 2004, 2010). They are expressed in leaves, stem, and roots of several plants species belonging to families like Rubiaceae, Violaceae, Cucurbitaceae, and Fabaceae (Craik et al. 2004). Cyclotides have two characteristic features. First is their N and C terminal moiety fused to form cyclic backbone. Secondly they have cysteine knot motif made up of six conserved cysteine residues (Craik et al. 2004). Presence of these features makes these proteins thermal, chemical, and enzymatic stable and resistant to protease (Craik et al. 2004). Kalata
B1 was the first cyclotides isolated from African plant *Oldenlandia affinis* (Gran 1973).

Recently Gao et al. (2010) tested a panel of chemically synthesized kalata B1 analogues with varying the amino acid sequence against dengue NS2B-NS3 protease. Their search revealed a cyclopeptide whose two full oxidized forms were able to inhibit of dengue viral NS2B-NS3 protease. Inhibition was substrate specific and competitive in nature (Gao et al. 2010) make it reliable candidate for the design of stable pharmaceuticals against dengue.

**Medicinal Plant with AntiRdRp activity**

NS5 is a multifunctional protein with RNA depend RNA polymerase (RdRp) and methyltransferase activity. RdRp is unique to the virus (as it is absent in humans) and thus a potential target for drug discovery against the virus. Recently, Allard and colleagues during in vitro screening of New Caledonian (world’s smallest yet diverse hot spot) plants they found several species with significant AntiRdRp activity against dengue virus. Out of several species, they choose to perform chemical analysis of *Cryptocarya chartacea*. Results led to isolation of several new mono- and falvanones named chartaceones A-F (1-6), along with pinocembrin. Using plaque reduction assay they discovered that dialkylated flavanone; chartaceones C-F (3-6) has the most significant NS5 RdRp inhibitory effect (Allard et al. 2011).

**Virus assembly inhibitors**

Dengue virus assembly occurs at the endoplasmic reticulum (ER) (Chambers et al. 1990). In the ER pRM and Envelope proteins undergo modification in which an oligosaccharide (14 residue (Glc)3(Man)9(GlcNAc)2) is added to the specific asparagines residues. Further, this residue is sequentially modified by ER localized enzyme called α-glucosidase. This step is crucial for proper folding, assembly and secretion of the virus (Figure 3B). Glucosidase inhibitors have been shown to interfere with the dengue virus growth.

Castanospermine, a natural alkaloid (a plant secondary metabolite) had reported to have α-glucosidase inhibitory action. It is extracted from the black bean or Moreton Bay chestnut tree (*Castanospermum australae*). Courageot and colleges have demonstrated the molecular basis of effect of inhibitory effect of Castanospermine/ deoxyxojirimycin (isolated from leaves of *Morus multicaulis*) on dengue virus serotype 1 (in vitro). Their finding suggests that this alkaloid may disrupt the proper interaction of prM and Envelope protein which is crucial for virus assembly (Courageot et al. 2000). In follow up study (Whitby et al. 2005) showed that Castanospermine is effective against all serotype of Dengue (DENV 1-4) both in vitro as well as in vivo (mice). Further they tested the effect of this alkaloid against two clinically important members of Flaviviruses viz. West Neil Virus (WNV) and Yellow Fever Virus (YFV). Their results indicate that Catanospermine had almost no effect on WNV and mild inhibitory effect on YFV. Virus growth inhibition was shown by plaque assay (Huh7 cells and BHK-21 cells) and flow cytometric assays (Whitby et al. 2005). Further they validated their findings in mice infection model. Interestingly, the compound is safe in mice and protects them specifically and efficiently against all DENV serotypes (Whitby et al. 2005).

Synthetic analogues of this glucosidase inhibitor have been shown to have protective effect against dengue infection in animal model (mice) (Chang et al. 2011). List of natural alkaloids with anti glucosidase activity is quite long. For example Calystegins an alkaloid extract from roots of naturally growing *Convolvulus arvensis*, had potent anti glucosidase activity (Molyneux et al. 1993). However there is no study so far (to our knowledge) where its inhibitory potential has been demonstrated against dengue virus.

**Unclassified Replication inhibitors: Anti-dengue Medicinal Plants**

There are several other plant species which inhibit dengue virus growth. But their precise mechanism of action has not been elucidated yet. A study by Sánchez et al. (2000) reported the effect of flavonoids extracted from the Mexican plants *Tephrosia madrensis*, *Tephrosia viridiflora*, and *Tephrosia crassifolia*. Out of
In a preliminary study by Klawikkan et al. (2011) investigated the in vitro anti-dengue activity from ten Thai medicinal plants (Klawikkan et al. 2011). Plants were extracted with dichloromethane followed by ethanol and tested against DENV2 in Vero cells. Cytotoxic effect of plant extract was determined by MTT assay (Mosmann, 1983) and reduction in virus growth was shown by plaque reduction assay. Their results indicate that out of ten, four medicinal plants viz. Rhizophora apiculata Blume., Flagellaria indica Linn., Cladogynos orientalis Zipp. and Houttuynia cordata Thunb exhibited potent anti-viral effect against DENV-2 (Klawikkan et al. 2011).

Very recently, Tang et al. (2012) in their primary study investigated the inhibitory effect of standardized methanic extracts of six medicinal plants viz. Andrographis paniculata, Citrus limon, Cymbopogon citratus, Momordica charantia, Ocimum sanctum and Pelargonium citrosum on dengue virus serotype 1 (DENV-1) in cell culture (Vero E6 cells). Using cytopathic inhibition and MTT assay they demonstrate that A. paniculata has the most anti-viral inhibitory effects followed by M. charantia. However, methanic extracts were tested against only one serotype of Dengue (DENV-1) thus study needs further extension (Tang et al. 2012).

Vector control

Current mosquito control strategies include the use of aerial sprays (toxicants) repellents, larvicides, insecticides as well as mosquito nets for personal protection (WHO 2009). Aerial toxicants against A. aegypti do not yield significant results because this mosquito is highly domesticated and many adults rest indoors in hidden places such as closets (WHO 2009).

It is worth mentioning here that efforts have been made to generate genetically modified (GM) mosquito in which gene has been incorporated into mosquito that kills the insect at the larval stage of its life cycle (Figure 2). The GM mosquito has been developed by scientists at Oxford biotechnology company Oxitec (Enserink, 2010). Gene is functionally active at larvae state and subsequently kills insect at the larval stage of its life (Figure 2). Speculation
about success of this approach is quite promising. Several reports (Fallatah and Khater 2010) are present where Larvicidal activity of plant extracts has been demonstrated has been summarized in table 2.

**Table 2:** Few examples of medicinal plants with larvicidal activities against mosquito vector responsible for transmission of Dengue.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Inhibitory compound</th>
<th>Inhibitory activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anacardium occidentalis, Copaifera langsdorffii, Carapa guianensis, Cymbopogon winterianus and Ageratum conyzoides</strong></td>
<td>Extracts and oils</td>
<td>Larvicidal activity against <em>Aedes aegypti</em></td>
<td>de Mendonça et al. (2005)</td>
</tr>
<tr>
<td><strong>Carum carvi, Apium graveolens, Foeniculum vulgare, Zanthoxylum limonella and Curcuma ze-dooaria</strong></td>
<td>Essential oil</td>
<td>Larvicidal activity against <em>A. aegypti</em></td>
<td>Pitasawat et al. (2007)</td>
</tr>
<tr>
<td><strong>Solanum villosum</strong></td>
<td>Berry extract</td>
<td>Larvicidal activity against <em>Stegomyia aegypti</em></td>
<td>Chowdhury et al. (2008)</td>
</tr>
<tr>
<td><strong>Combretum collinum</strong></td>
<td>Shoot bark extract</td>
<td>Larvicidal activity against <em>A. aegypti</em></td>
<td>Odda et al. (2008)</td>
</tr>
<tr>
<td><strong>Azadirachta indica and Pongamia glabra</strong></td>
<td>Herbal formulation (PON-NEEM)</td>
<td>Larvicidal, ovicidal and oviposition deterrent activities against <em>A. aegypti</em> and <em>A. albopictus</em></td>
<td>Maheswaran and Ignacimuthu (2011)</td>
</tr>
<tr>
<td><strong>Nyctanthes arboristis, Catharanthus roseus Boe-ninninghusenia albiflora, Valeriana hardwickii and Eupatorium odoratum</strong></td>
<td>Leaf extract</td>
<td>Larvicidal properties against <em>Anopheles stephensi</em>, <em>A. aegypti</em> and <em>Culex quinquefasciatus</em></td>
<td>Alam et al. (2011)</td>
</tr>
<tr>
<td><strong>Citrus limetta</strong></td>
<td>Extracts from the peels</td>
<td>Larvicidal <em>Aedes aegypti</em>, and malarial vector, <em>Anopheles stephensi</em></td>
<td>Kumar et al. (2012)</td>
</tr>
<tr>
<td><strong>Acalypha alnifo- lia</strong></td>
<td>Leaf extract</td>
<td>Larvicidal activity against <em>A. stephensi</em>, <em>A. aegypti</em>, <em>C. quinquefasciatus</em></td>
<td>Kovendan et al. (2012)</td>
</tr>
<tr>
<td><strong>Delonix elata</strong></td>
<td>Leaf and seed extracts</td>
<td>Larvicidal and ovicidal activities against of <em>A. stephensi</em> and <em>A. aegypti</em></td>
<td>Marimuthu et al. (2012)</td>
</tr>
</tbody>
</table>

**Anti dengue herbal patents**

Plants are rich source of essential oils (EOs), glyceridic oils with mosquito repellent activities (Pohlit et al. 2011a&b). Several plants derived mosquito repellent products have been patented which have been extensively reviewed elsewhere (Pohlit et al. 2011a&b).

However, not much patents are available where plant extract has been patented against all serotypes of Dengue virus (DENV1-4). To our knowledge only one such patent is available where anti Dengue activity of plant *Cissampelos parier* extracts have been patented by interdisciplinary team of Indian Scientists from International Centre for Genetic Engineering and Biotechnology, New Delhi, India , Dalichi Life Science Research Centre in India (DSIN) (earlier known as Ranbaxy Research Laborato-
extract also showed analgesic/anti-inflammatory activity along with anti-dengue activity (Raut et al. 2012). This in future could lead to development of cost effective, safe, and effective drug against Dengue infection.

Engineering biosynthetic plant pathways in microorganism

The secondary metabolites of higher plants include diverse chemicals, such as alkaloids, isoprenoids, and phenolic compounds (phenylpropanoids and flavonoids) which are generated from primary metabolites such as amino acids or acetyl-CoA with the help of biosynthetic pathways unique to plants. Although these compounds have variety of medicinal properties however, amount of bioactive compound available after extraction from plant is quite low. In the era of synthetic biology, several efforts have been made in which these secondary metabolites are produced in large quantities in engineered microorganisms by incorporating genes responsible for synthesis of these metabolites into microorganisms (Nakagawa et al. 2011; Chemler et al. 2008). These finding further raises hopes for the development of cost effective drug against dengue.

Conclusion

Dengue infection has been re-emerging as a serious life threat with increase in the infection cases each year. It is becoming one of the major health issues across the tropical and the subtropical belt. Various strategies have been adapted to develop an effective vaccine or drug against Dengue virus. However, till date there is no licensed vaccine available in the market. There is an urgent need to find alternate solution to combat dengue infection. Medicinal plants have been utilized for the treatment of several human diseases. Several studies have been made where anti Dengue potential of medicinal plants has been reported. However, they are still far from success. There is need of extensive and fruitful networking among academic research groups, clinicians, and industries throughout the globe so that Ethnobotanical knowledge can be circulated and finally converted into effective drug against Dengue (DENV 1-4).

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