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Review Article

Dengue and Its Phytotherapy: A Review

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Abstract

Dengue fever causes mortality and morbidity around the world, specifically in the Tropics and subtropic regions, which has been of major concern to governments and the World Health Organization (WHO). Dengue fever or dengue hemorrhagic fever is the most prevalent viral disease caused by dengue virus a family of flavivirus transmitted through *Aedes aegypti* mosquito. Approximately 2.5 billion people word wide affected by this virus. As there are no synthetic drugs available, now it is need to focus on medicinal plants which are considered to be effective, safer and non-toxic. Hence the search for new anti-dengue agents from medicinal plants has assumed more urgency than in the past. Medicinal plants have been used widely to treat a variety of vector ailments such as malaria. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs. This article reviews potential anti-dengue activities from plants distributed around the world. Current studies show that natural products represent a rich potential source of new anti-dengue compounds. Further ethno botanical surveys and laboratory investigations are needed to establish the potentials of identified species in contributing for dengue control.

1. INTRODUCTION

Dengue fever is caused by the arthropode-borne flavi virus named dengue virus (DENV), transmitted by the *Aedes aegypti* mosquito[1]. To date, four antigenically related but distinct virus serotypes (DENV-1, 2, 3 and 4) have been identified as belonging to the genus Flavi virus in the Flaviviridae family[2– 4]. Infection with one DENV serotype produces only specific antibody against that serotype. When antibody from the first infection is neutralized, secondary infections by other serotypes can cause more serious infection [5]. Although DENV-2 is known to be more lethal than other serotypes [6], some studies have revealed that primary infection with DENV-1 or DENV-3 always results in more dangerous disease than infection with DENV-2 or DENV-4[3,7]. In recent years, the current dengue epidemic has become a focus of international public health awareness. Unlike malaria, which is more prevalent in remote areas, cases of dengue are distributed mostly in urban and sub-urban areas[8, 9]. This has made the epidemic more lethal as an outbreak is difficult to control due to highly populated areas in cities. Types of DENV infection include mild fever known as dengue fever (DF), which constitutes about 95 % of cases, and a more serious type known as dengue hemorrhagic fever and/or dengue shock syndrome (DHF/DSS, 5% of cases)[10, 11]. Recovery from first type of infection provides lifelong immunity; however, it affords only half protection from subsequent viral infection that ultimately results in the risk of DHF. Most dengue infections are characterized by non-specific symptoms including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness and rash[12, 13].

2. HISTORY OF DENGUE VIRUS

Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice[14] and at Calcutta (now Kolkata) in 1944 from serum samples of US soldiers[4, 15]. The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964[16-18]. The first major epidemic of the DHF occurred in 1953-1954 in Philippines followed by a quick global spread of epidemics of DF/DHF[19]. DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in various parts of India since 1988[20-22] The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi[23] and Lucknow [24] and then it spread to all over the country[25].

3. EPIDEMIOLOGY OF DENGUE FEVER

International travel, increasing human population[26,27] and urbanisation create suitable conditions for the mosquito vector *Ae. aegyti*, and thus spread the virus to new areas, causing major epidemics[13,28,29]. Dengue epidemics are endemic in over 100 countries in Africa, America, Eastern Mediterranean, Southeast Asia and Western Pacific, with Southeast Asia and the Western Pacific being the region's most affected[13,30-32]. The first case of DHF was discovered in the 1950s in Thailand and the Philippines[4], where the first two DENV serotypes were identified, followed by the third and fourth serotypes in 1954[26]. Since then, DHF has recorded major cases resulting in hospitalization and death among children in regions stretching from Asia to Africa and the Pacific[4]. Approximately 2.5 billion people, or half the world's population[26], are now at risk of Dengue, and 50 million infections globally occur annually[4]. Over 100 million cases of DF and at least 500,000 cases of DHF[33] and approximately 18,000 deaths may occur each year[34]. Despite its lethal consequences, the staggering numbers of those affected are increased by the fact that, at present, there is no specific antiviral treatment or vaccine for DF[3]. Early diagnosis and strict hospitalization often save the life of patients with DHF [3,4,10]. Efforts to combat the vector have been undertaken by regulatory bodies in an attempt to tackle this problem by awareness campaigns and vector control[28]. Other strategies include the use of plants with bioactive substances that have toxic properties to the vector or insecticidal properties[32]. Clearly, development of antiviral drugs and vaccines is needed in order to support these programs. Moreover, a safe, low-cost, and effective vaccine to control DENV would be needed, especially in the most affected countries, which are poor[2,28]. Therefore, the search of highly selective but non-toxic

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antiviral compounds is urgently needed in view of the spread of dengue disease throughout the world[35].

4. GLOBAL DISTRIBUTION OF DENGUE FEVER

Guangdong province in China has become a major area with reported cases of dengue[36]. From 2000 to 2005, a total of 2,496 cases of dengue were recorded. The epidemic peaked in 2002. In Northern Thailand there were 13,915, 11,092, 6,147, 6,992 and 6,914 DF cases reported during the period 2002–2006[37]. Outbreaks of DF and DHF have been reported in India over the past four decades[38]. From 2001 to 2002, Delhi recorded a decline in cases of DF/DHF, with a total of 1,380 cases, but deaths decreasing from 53 cases (2001) to 35 cases (2002). However, outbreaks of DF cases rose sharply in 2003, with a total of 12,754 cases and 215 deaths.

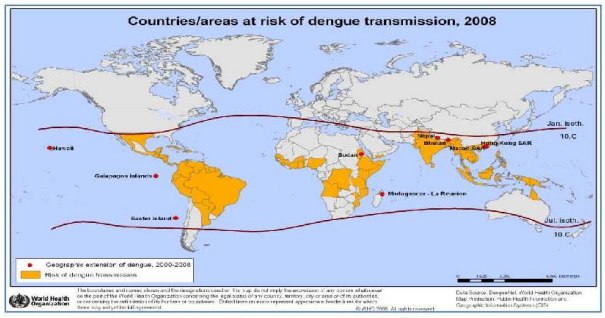

Figure 1: Showing the Geographic extension of Dengue

5. DENGUE FEVER IN INDIA

The epidemiology of dengue fevers in the Indian sub continent has been very complex and has substantially changed over almost past six decades in terms of prevalent strains, affected geographical locations and severity of disease.

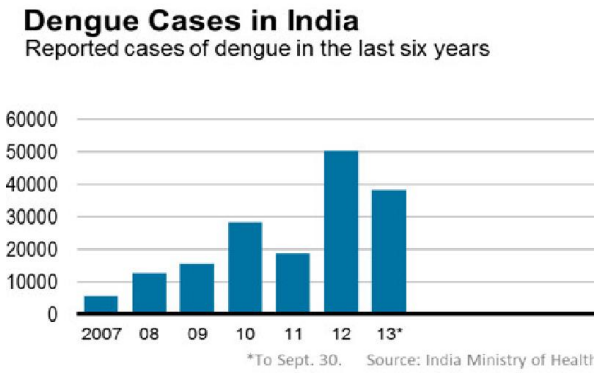

Figure 2: Showing the Dengue cases in India up to 2013

The very first report of existence of dengue fevers in India was way back in 1946.[39] Thereafter, for the next 18 years, there was no significant dengue activity reported anywhere in the country. In 1963-1964, an initial epidemic of dengue fever was reported on the Eastern Coast of India[40,41-45] it spread northwards and reached Delhi in 1967[46] and Kanpur in 1968[47,48]. Simultaneously it also involved the southern part of the country[49,50] and gradually the whole country was involved with wide spread epidemics followed by endemic/hyper endemic prevalence of all the four serotypes of DV. The epidemiology of dengue virus and its prevalent serotypes has been ever changing. The epidemic at Kanpur during 1968 was due to DV-422 and during 1969 epidemic; both DV-2 and DV-4 were isolated[51]. It was completely replaced by DV-2 during 1970 epidemic in the adjoining city of Hardoi[52]. Myers *et al* [49,53] had reported the presence of DV-3 in patients and *Ae. aegypti* at Vellore during the epidemic of 1966 while during the epidemic of 1968, all the four types of DV were isolated from patients and mosquitoes[54]. In another study Myers & Varkey[55] reported an instance of a third attack of DV in one individual. DV-2 was isolated during the epidemics of dengue in urban and rural areas of Gujarat State during 1988 and 1989[56]. Outbreaks of dengue occurred in Rajasthan by DV- 1 and DV-3[57], DV-3[58], Madhya Pradesh by DV-3[59], Gujarat by DV-2[56] and in Haryana by DV-2[60]. DV-2 was the predominant serotype circulating in northern India, including Delhi, Lucknow and Gwalior[23,24,61] while DV-1 was isolated during the 1997 epidemic at Delhi[62]. The phylogenetic analysis by the Molecular Evolutionary Genetics Analysis programme suggests that the 1996 Delhi isolates of DV-2 were genotype IV. The 1967 isolate was similar to a 1957 isolate of DV-2, from India, and was classified as genotype V. This study indicates that earlier DV-2 strains of genotype V have been replaced by genotype IV[63]. The Gwalior DV-2 viruses were classified into genotype-IV, and were most closely related to Delhi 1996 DV-2 viruses and FJ 10/11 strains prevalent in the Fujian State of China. However, two earlier Indian isolates of DV-2 were classified into genotype-V. Genotype V of DV-2 has been replaced by genotype IV during the past decade, which continues to circulate silently in north India, and has the potential to re-emerge and cause major epidemics of DF and DHF[64]. DV-2 has also been reported from southern India - in Kerala along with DV-3[65]. DV-3 has been isolated during the epidemics at Vellore in 1966[49,53] at Calcutta in 1983[66] and in 1990[21] at Jalore city, Rajasthan in 1985[58] at Gwalior in 2003 and 2004[67,68] and at Tirupur, Tamil Nadu in 2010[69].

6. PATHOPHYSIOLOGY OF DENGUE FEVER

Dengue infection is caused by bites of the female *Ae. aegypti* mosquito carrying Flavivirus. After a person is bitten, the virus incubation period varies between 3 and 14 days[3,30] after which the person may experience early symptoms such as fever, headache, rash, nausea, and joint and musculoskeletal pain[3,13]. This classic DF records temperatures between 39-40^{0}C and usually lasts 5–7 days[6]. During this period, the virus may get into the peripheral bloodstream and, if left untreated, can damage blood vessels and lymph nodes

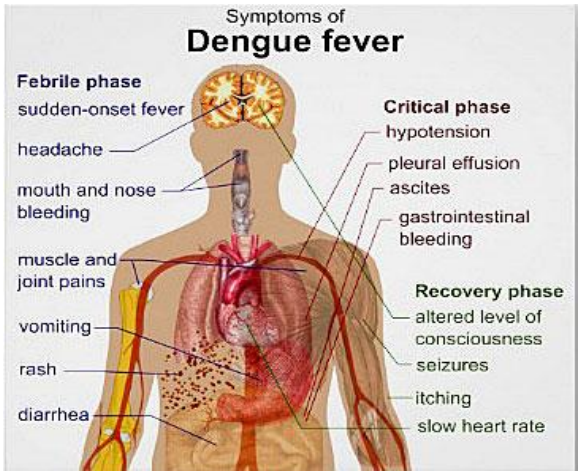

Figure 3: Showing symptoms of Dengue

resulting in DHF with symptoms such as bleeding from the nose, gums or under the skin[30]. DHF patients also have difficulty in breathing and severe development can lead to DSS. DSS can result in death if proper treatment is not provided. Aedes mosquitoes are small and black with white markings on the body and legs. Female mosquitoes need blood from biting humans or animals to produce live eggs. It takes 2–3 days for egg development. The principal vector of dengue (Ae. aegypti) has adapted well to the urban environment[26,29] and always breeds in stagnant containers. Eggs need moist conditions, and mature in 24–72 h[70]. Mosquito bites are the only route of DENV spread. The transmission of DENV is often from human to human through domestic mosquitoes[6]. An outbreak starts after a mosquito sucks the blood of a patient with DF/ DHF[70]. After being transmitted to a new human host by infected mosquitoes, the virus replicates in the lymph nodes and spreads through the lymph and blood to other tissues[6]. To identify a potential antiviral treatment for DENV, it is necessary to understand the life cycle of the virus.

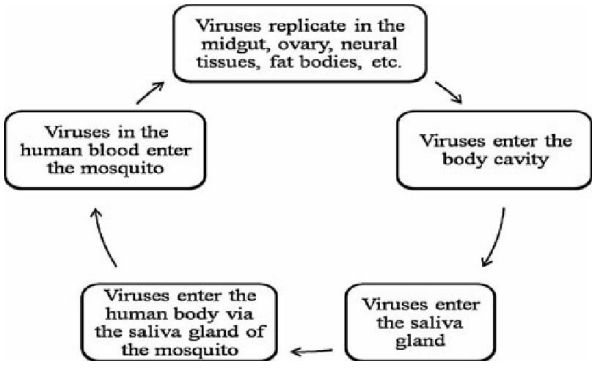

Fig. 4: Dengue virus transmission cycle

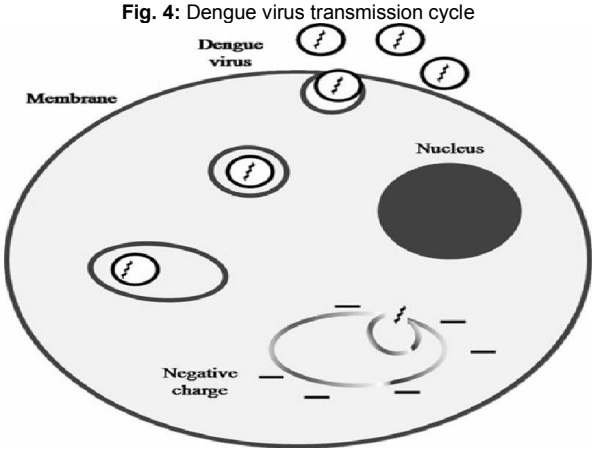

Fig. 5: Dengue virus infection cycle in cells

The dengue virion is a small particle with a lipoprotein envelope and an icosahedral nucleocapsid containing a positive single-stranded RNA genome [6,12,35]. Virus infection of the cell begins with binding to the host cell surface. It enters the cell by receptor-mediated endocytosis[27], with the cell membrane forming a sac-like structure known as an endosome. In the endosome, the virus penetrates deep into the cell until the endosome membrane acquires a negative charge, which allows it to fuse with the endosomal membrane to open a port for release of genetic material. At this point, the virus in the cell fluid starts to reproduce. Changes in the acidity of the secretory pathway during this viral journey travel play an important role in its maturation possible mechanisms and pathways in the treatment of dengue There are currently no specific treatments for dengue fever[34]. Only standard treatment for management of fever is given, i.e., nursing care, fluid balance, electrolytes and blood clotting parameters[30]. Patients with dengue fever will be treated symptomatically, for example, sponging, acetaminophen[9], bed rest and oral rehydration therapy, and if signs of dehydration or bleeding occurs the patients are usually hospitalized[6]. Aspirin should be avoided because it may cause bleeding[9]. Platelet count and Hematocrit should be measured daily from the suspected day of illness until 1–2 days after defervescence[9]. Current prevention of dengue by potential dengue vaccine and vector control is highly cost effective[34,71]. In addition, mosquito control programs are the most important preventive method[6]. However, these are difficult to implement and maintain[72]. Development of a vaccine for dengue is difficult since there are four closely related, but antigenically distinct, serotypes of the virus that can cause disease[6,73]. Infection by one serotype does not ensure protection of the patient from infection by the other three serotypes[27]. Therefore, if vaccine were produced for only one or two serotypes, the other serotypes would increase the risk of more serious illness[74]. Ribavirin has shown significant in vivo activity against RNA viruses; however, it exhibited only very weak activity against Flaviviruses[33]. A possible strategy in the treatment of dengue is to use chimeric tetravalent vaccines that show high neutralizing antibody against all dengue serotypes[9,27]. Studies on the development of tetravalent vaccines are ongoing in Thailand and these should be available in the near future[6]. In addition, recombinant vaccines against capsid, premembrane and envelope genes of DENV-1, 2 and 3 inserted into a copy of a DNA infectious clone of DENV-2 are being developed and are currently undergoing clinical trials[75].

7. PLANTS TRADITIONALLY USED TO TREAT DENGUE

According to a World Health Organization (WHO) fact sheet dated December 2008, 80 % of the population in some Asian and African countries depends on traditional medicine as their primary health care due to economic and geographical constraints[76]. Natural products have become the main source of test material in the development of antiviral drugs based on traditional medical practices[77]. Traditional medicines are based on knowledge, experience and practices based on indigenous cultural beliefs and knowledge, and are used to maintain health, prevent, treat and diagnose physical or mental illness[76]. Traditional medicinal plants have been reported to have antiviral activity[76,78,79] and some have been used to treat viral infections in animals and humans. To date, 31 different species have been found to have the potential to treat dengue; some of these have not yet been investigated scientifically. In the Philippines, Euphorbia hirta, known locally as ‘‘tawa–tawa’’, is used in folk medicine to cure dengue fever by people in rural areas[80]. Practitioners of traditional medicines believe that decoction of tawa–tawa leaves can reverse viral infection and prevent the fever from moving into critical stages, although there are no scientific studies proving its effectiveness[81]. Sometimes, tawa–tawa is prepared together with papaya leaves since papaya leaf extract has a function as an antibiotic to cure fever. While papaya leaf extract kills the bacterial infection that caused the fever, tawa–tawa extract prevents bleeding. In addition, unpublished research has found that Psidium guava leaves are a good way to increase platelets, thus helping to avoid bleeding[82]. A water decoction of guava leaves contains quercetin, which acts to inhibit the formation of enzyme mRNA in the virus[83].

7.1 Overview of studies on plant species used against Dengue

The use of herbal-based medicine and medicinal plants to treat many diseases is growing worldwide as they has few or no adverse effects. The following sections describe some species of medicinal plants from various families that have been investigated for anti-dengue activity. In addition, we describe species used as traditional treatment for dengue together with their isolated compound. Alternanthera philoxeroides Alternanthera philoxeroides belongs to family Amaranthaceae. A. philoxeroides is also called ‘‘Alligator Weed’’, and is an immersed aquatic plant. It originated from South America but is currently invading Australia. The effect of A. philoxeroides extracts against dengue virus was investigated in vitro[84]. An MTT assay was carried out to determine the cytotoxicity of A. philoxeroides on C6/36 cell lines. Coumarin extract of A. philoxeroides showed lowest toxicity on cells (TD50 = 535.91), whereas a petroleum ether extract of A. philoxeroides had the strongest inhibitory effect on dengue virus (ED50 = 47.43). *Andrographis paniculata, Andrographis paniculata* belongs to family Acanthaceae. It is an erect annual herb native to India and Sri Lanka and cultivated widely in Southern and Southeastern Asia. In Malaysia, it is called ‘‘Hempedu Bumi’’, which has a bitter taste. The maximum nontoxic dose (MNTD) of methanolic extract of *A. paniculata* against Vero E6 cells in vitro was investigated[7]. *A. paniculata* recorded the maximal dose, which was not toxic to cells at 0.050-1. The methanolic extract of A. paniculata showed the highest antiviral inhibitory effect on DENV-1 by antiviral assay based on cytopathic effects.

7.2 *Alternanthera philoxeroides*

Alternanthera philoxeroides [85] (Commonly called Alligator Weed) as is perennial aquatic plant belonging to Amaranthaceae family. Jiang et al (2005)[86] investigated the antiviral activity of four extracts (petroleum ether, ethyl acetate, ethyl ether and coumane of *A. philoxeroides.* Their results indicated that all extracts posses anti-dengue activity but highest inhibition of dengue virus was observed with petroleum ether extract.

7.3 *Andrographis paniculata*

Andrographis paniculata[87,88] belonging to family Acanthaceae, is an erect herb which is extremely bitter in taste. This plant has been effectively used as traditional medicine for centuries. Chemically, *A.*

paniculata has major constituents, like lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. It also effectively used in treating symptoms of upper respiratory tract infections. Tang etal., (2012)[89] has reported in vitro studies of antiviral activity of methanolic extract of *A. paniculata* on dengue fever. In this preliminary screening study for anti-dengue agent, methanolic extract of *A. paniculata* was found to have high potential to be an anti-dengue agent, particularly towards DENV-1 serotype.

7.4 *Azadirachta indica*

Azadirachta indica belongs to the family Meliaceae. It is fast-growing tree with a final height in the range of 15–20 m. It is native to India and Pakistan and grows throughout tropical and semi-tropical regions. The in vitro and in vivo inhibitory potential of aqueous extract of *Azadirachta indica* (Neem) leaves on the replication of DENV-2 was evaluated[8]. Cytotoxicity studies were carried out to determine the MNTD in a virus inhibition assay. The aqueous extract of neem leaves (NL) completely inhibited 100–10,000 tissue culture infective dose (TCID)50 of virus as indicated by the absence of cytopathic effects at its maximum non-toxic concentration of 1.897 mg mL^{-1}. An in vivo study on the inhibitory effects on virus of NL aqueous extract in day-old suckling mice was carried out by intracerebral inoculation. It was shown that the aqueous extract inhibited the virus at nontoxic doses in the range of 120–30 mg mL-1 as indicated by the absence of 511-bp dengue group specific amplicons upon RT-PCR.

7.5 *Boesenbergia rotunda*

Boesenbergia rotunda belongs to family Zingiberaceae. It is a medicinal and culinary herb known as Chinese ginger. It is found throughout China and Southeast Asia. The activity of some compounds extracted from B. rotunda for the inhibition of dengue virus protease has been tested on DENV-2[90]. The cyclohexenyl chalcone derivatives of B. rotunda, 4-hydroxypanduratin A (1) and panduratin A (2) showed good competitive inhibitory activities towards DENV-2 NS3 protease with Ki values of 21 μM and 25 μM, respectively. The small value of Ki shows the potential of 4-hydroxypanduratin A to inhibit DENV-2 NS3 protease in vitro.

7.6 Boerhaavia diffusia

Punarnava is the telugu name of *Boerhaavia diffusia*[91] belonging to the family of Nyctaginaceae. It is distributed all over the world like Africa, Asia, North America, South America, and South Pacific. Boerhaavia diffusia has found to shown various important biological activities like antibacterial, anti-oxidant, antidiabetic, anti-diuretic and anti-inflammatory etc. The root is mainly used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, and abdominal pain. Priyank Bharati and Rajashree Sinha[92] have studied the anti-dengue effect of stems of Tinospora cardifolia (Wild) Miers (10 gm) and the plant of Boerhaavia diffusa Linn (10 gm). Anti-dengue effect was evaluated by giving the Ayurvedic mixture consisting Tinospora cardifolia and Boerhaavia diffusa to dengue patients 2- 3 times a day.

7.7 *Carica papaya*

Carica papaya belongs to family Caricaceae. It is an erect, fast-growing and unbranched tree or shrub indigenous to Central America and cultivated in Mexico and most tropical countries for its edible fruits. *C. papaya* leaf has been used traditionally in the treatment of DF[82]. The leaf has been investigated for its potential against DF. The aqueous extract of leaves of this plant exhibited potential activity against DF by increasing the platelet (PLT) count, white blood cells (WBC) and neutrophils (NEUT) in blood samples of a 45-year-old patient bitten by carrier mosquitoes[9]. After 5 days of oral administration of 25 ml aqueous extract of *C. papaya* leaves to the patient twice daily, the PLT count increased from 55 9 103/μL to 168 9 103/μL, WBC from 3.7 9 103/ μL to 7.7103/μL and NEUT from 46.0 to 78.3 %. Increased platelets could lead to reduced bleeding, thus avoiding progression to the severe illness of DHF.

7.8 *Castanospermum austral*

Castanospermum belongs to the Fabaceae family and has only one species -*Castanospermum australe*, commonly referred to as the Black Bean. Whitby et al. (2005)[93] has investigated anti viral activity of castanospermine, is a natural alkaloid derived from the tree *C. australae* by in Vitro assay. Castanospermine has showed good inhibitory anti-dengue activity over a broad range of doses from 10 to 250 mg/kg/day. This investigation reveals that castanospermine acts as an ER α-glucosidase I inhibitor and reduces infection of a subset of enveloped RNA and DNA viruses in vitro. Studies of its mechanism of action suggest that castanospermine may disrupt folding of some viral proteins by preventing the removal of the terminal glucose residue on N-linked glycans in dengue virus.

7.9 *Chondrus crispus*

Chondrus crispus commonly called as carrageen moss is a species of red algae. It is abundant in rocky shores and tide pools of Ireland and coast of Europe. *Chondrus crispus*, consisting polysaccharide carrageen as active constituent. Carrageenans are effective in treatment against viral infections of common cold. Talarico et al (2007)[94] has reported that carrageen and other sulfate polysaccharides were effectively inhibited the dengue virus 2 infection where they were inhibiting virus entry.

7.10 *Cissampelos pareira*

Cissampelos pareira[95] is also known as velvet leaf, belongs to family of Menispermaceae. This plant is widely distributed worldwide and has been used in the treatment of ulcers, diarrhea, muscle inflammation and rheumatism. Earlier investigations on roots of *Cissampelos pareira* revealed that it contain alkaloids hyatin hyatinin, haytidine and bebeerines. A chalcone and flavones dimer was isolated from the aerial parts of the plant named as cissampeloflavone. Bhatnagar and Co-workers (2012)[96] patented anti-dengue activity of extract of aerial parts of *Cissampelos pareira.* Their investigations related to anti-dengue activity of *Cissampelos pareira* extracts and a pharmaceutical compounds were also provided comprisning *Cissampelos pareira* extracts. Methanolic extractx of *Cissampelos pareira* showed anti-viral activity against all types of dengue virus in conventional assay with PRNT50 values in the range of 1.2-11.1 μg/mL.

7.11 *Cladogynos orientalis*

Cladogynos orientalis belongs to family Euphorbiaceae. It is a white-stellate-hairy shrub about 2 m high found in Southeast Asia, Malaysia and Thailand. The in vitro activity of *Cladogynos orientalis*—a Thai medicinal plant—against dengue virus was evaluated[2]. The dichloromethane ethanol extract of *C. orientalis* was tested for anti-dengue activities against DENV-2 in Vero cells by the MTT method. The results showed that the ethanol extract of *C. orientalis* at a concentration of 12.5 μg mL^{-1} exhibited inhibitory activity on DENV-2 with 34.85 %. In addition, *C. orientalis* at a concentration of 100 μg mL^{-1} exhibited an inactivated viral particle activity of 2.9 %.

7.12 *Cladosiphon okamuranus*

Cladosiphon okamuranus belongs to family Chordariaceae. It is brown seaweed found naturally in Okinawa, Japan. A sulfated polysaccharide named fucoidan[3] from *Cladosiphon okamuranus* was found to potentially inhibit DENV-2 infection[97]. The virus infection was tested in BHK-21 cells in a focus-forming assay. Fucoidan reduced infectivity by 20 % at 10 lg mL^{-1} as compared with untreated cells. However, a carboxy-reduced fucoidan in which glucuronic acid was converted to glucose attenuated the inhibitory activity on DENV2 infection.

7.13 *Cryptonemia crenulata*

Cryptonemia crenulata belongs to family Halymeniaceae. It is a marine species found throughout the Atlantic Islands, North America, Caribbean Islands, Western Atlantic, South America, Africa, Indian Ocean Islands, Southeast Asia and Pacific Islands. The sulfated polysaccharides from *Cryptonemia crenulata*, i.e., galactan (4), were selective inhibitors of DENV-2 multiplication in Vero cells with IC50 values of 1.0 lg mL^{-1}, where the IC50 values for the reference polysaccharides heparin and DS8000 were 1.9 and 0.9 lg mL^{-1}, respectively[98]. However, the compound has lower antiviral effect against DENV-3 and DENV-4, and was totally inactive against DENV-1. The inhibitory effect of C2S-3 against DENV-2 was slightly higher when treatment was by adsorption (EC50 = 2.5 ± 0.1 μg mL^{-1}) with respect to treatment only during internalization (EC50 = 5.5 ± 0.7 μg mL^{-1})[1]. Thus, the inhibitory effect was increased when C2S-3 was included at both stages of adsorption and internalization.

7.14 *Cymbopogon citratus*

Cymbopogon citratus belongs to family Poaceae. It is a grass species known as lemon grass and is a tropical plant from Southeast Asia. The antiviral activity of *Cymbopogon citratus* was determined based on cytopathic effects shown by the degree of inhibition of DENV-1 infected Vero E6 cells[7]. The methanolic extract of *C. citratus* showed a slight inhibition effect on DENV-1. This result was further confirmed with an inhibition assay by the MTT method. However, *C. citrates* showed no significant inhibition. Moreover, *C. citratus* showed the lowest of MNTD at concentration of 0.001 mg mL^{-1}. *C. citratus* was found to be quite a cytotoxic plant as it showed maximum cytotoxicity at 0.075 mg mL^{-1}.

7.15 *Euphorbia hirta*

Euphorbia hirta belongs to family Euphorbiaceae. It is a common weed in garden beds, garden paths and wastelands and is found throughout Java, Sunda, Sumatra, Peninsular Malaysia, the Philippines and Vietnam. The water decoction of leaves from *Euphorbia hirta*, locally known as gatas–gatas, is used in the Philippines as a folk medicine to treat DF[81]. Internal haemorrhaging will stop and dengue fever will be cured after 24 h. However, the mechanism of action is still unknown and the antiviral properties and its ability to increase blood platelets are currently investigated. The tea obtained from boiled leaves of *E. hirta* is used to cure DF[80].

7.16 *Flagellaria indica*

Flagellaria indica belongs to family Flagellariaceae. It is robust perennial climber that grows in many of the tropical and subtropical regions of the Old World, India, Southeast Asia, Polynesia and Australia. *Flagellaria indica* was investigated for its anti-dengue properties in Vero cells[2]. The antiviral assay results show that 45.52 % inhibition of DENV-2 was observed in vitro in the presence of 12.5 µg mL^{-1} of ethanol extract of the plant. By conducting MTT assays, the cytotoxicity of *F. indica* was determined. The CC50 of ethanol extract of *F. indica* were 312 gmL^{-1}. Thus, this study indicates that *F. indica* has a significant potential effect on DENV.

7.17 *Gastrodia elata*

Gastrodia elata has been known as famous and important chinese medicinal herb belonging to family Orchidaceae. This traditional Chinese herb has been used to treat various diseases like stroke, rheumatism, insomnia, alzheimer's disease, depression, convulsions, neuronal diseases, fungal infections etc. Chemical analytical studies revealed that this plant contains nine kinds of phenolic compounds, and sixteen kinds of amino acids which are beneficial to health. Qui H (2007) and Tong (2010) et al.,[99] has isolated some D-glucans from *Gastrodia elata* and sulfated derivatives were prepared and they were investigated anti-dengue activity against dengue 2 virus. These sulfated D-glucan derivatives were strongly interfering with the dengue 2 virus infections with an EC(50) value of 0.68+/-0.17 µg/mL, mainly interfered with virus adsorption, in a very early stage of the virus cycle.

7.18 *Gymnogongrus griffithsiae*

Gymnogongrus griffithsiae belongs to family Phyllophoraceae. It is a red seaweed found in Ireland, Europe, Atlantic Islands, North America, South America, Caribbean Islands, Africa, Southwest and Southeast Asia and Australia and New Zealand. The inhibitory properties against DENV-2 of the sulphated polysaccharide from *Gymnogongrus griffithsiae*, kappa carrageenan (5) was evaluated in Vero cells[98]. The compound effectively inhibits DENV-2 multiplication at the IC50 value of 0.9 $µgmL^{-1}$, which is the same as the IC50 value for the commercial polysaccharides DS8000. However, the compound has lower antiviral effect against DENV-3 and DENV-4, and was totally inactive against DENV-1.

7.19 *Gymnogongrus torulosus*

Gymnogongrus torulosus belongs to family Phyllophoraceae. It is red seaweed found in Australia and New Zealand. *Gymnogongrus torulosus* was investigated for its in vitro antiviral properties against DENV-2 in Vero cells[100]. Galactan (4) extracted from this plant was active against DENV-2, with IC50 values in the range of 0.19–1.7 µg mL^{-1}.

7.20 *Hippophae rhamnoides*

Hippophae rhamnoides belongs to family Elaeagnaceae. It is a deciduous shrub occurring throughout Europe including Britain, from Norway south and east to Spain, and in Asia to Japan and the Himalayas. The anti-dengue activity of extracts of *Hippophae rhamnoides* leaves was investigated against dengue virus type-2 (DENV-2) in infected blood-derived human macrophages[11]. The findings showed that cells treated with *H. rhamnoides* leaf extracts was able to maintain cell viability of dengue-infected cells on par with Ribavirin, a commercial anti-viral drug along with a decrease and increase in TNF-a and IFN-c, respectively. Moreover, *H. rhamnoides* leaf extract proved its anti-dengue activity as indicated by a decrease in plaque numbers after the treatment of infected cells.

7.21 *Houttuynia cordata*

Houttuynia cordata belongs to family Saururaceae. It is herbaceous perennial flowering plants growing between 20 and 80 cm, and is native to Japan, Korea, Southern China and Southeast Asia. Ethanol extract from Houttuynia cordata revealed an anti-dengue activity with 35.99 % inhibition against DENV-2 in Vero cells at a concentration of 1.56 µg mL^{-1} [2]. Aqueous extract of *H. cordata* showed effective inhibitory action against DENV-2 through direct inactivation of viral particles before infection of the cells[5]. A concentration of 100 µg mL^{-1} also effectively protects the cells from viral entry and inhibits virus activities after adsorption. HPLC analysis of *H. cordata* extract indicated that hyperoside[6] was the predominant bioactive compound, and was likely to play a role in this inhibition.

7.22 *Hippophae rhamnoides*

Hippophae rhamnoides is a deciduous shrub belongs to Elaeagnaceae which has medicinal and nutritional values. The leaves and fruits of this plant is rich in vitamin A,B,C,E,K, flavanoids, lycopene, carotenoids and phytosterols[101]. Medicinally it was found to possess anti-oxidant, immune modulating activity, anti-cancer, anti inflammatory, anti-bacterial, anti-viral and wound healing activities. The anti-dengue activity[102] of *Hippophae rhamnoides* was investigated by Mounika Jain et al., (2008). The leaf extract of this plant was evaluated for anti-dengue activity in Dengue type 2 virus infected blood-derived human macrophages as the primary targets. This study showed that this extract was able to maintain cell viability of dengue infected cells and increases in TNF-α and IFN-γ respectively.

7.23 *Kaempferia parviflora*

Kaempferia parviflora is also known as krachai Dam, a thai traditional herb belonging to Zingiberaceae. Leaves and stem of this plant are used traditionally to treat many viral infections. Main chemical constituents of *Kaempferia parviflora* are borneol and flavanoids. Previous investigations reported that it has various activities like anti-ulcer, anti-allergic, anti-fungal, antimycobacterial etc. Recently, it has demonstrated very good activity against Dengue type 2 virus. Phurimask et al.,(2005)[103] has studied virucidal activity of leaves and stem extracts of *Kaempferia parviflora* against dengue virus type 2. It was suggested that some of the bioactive compounds in Kaempferia parviflora inactivates the Dengue type 2 virus particles.

7.24 *Leucaena leucocephala*

Leucaena leucocephala belongs to family Fabaceae. It is a species of Mimosoid tree indigenous throughout Southern Mexico and Northern Central America and the West Indies from the Bahamas and Cuba to Trinidad and Tobago. Galactomannans (7) extracted from seeds of *Leucaena leucocephala* have demonstrated activity against yellow fever virus (YFV) and DENV-1 in vitro and in vivo[12]. Galactomannans are polysaccharides consisting of a mannose backbone with galactose side groups, more specifically their structure consists of a main chain of (1 - 4)-linked b-D-mannopyranosyl units substituted by a-D-galactopyranosyl units[104]. *L. leucocephala* show protection against death in 96.5 % of YFV-infected mice. In vitro experiments with DENV-1 in C6/36 cell culture assays showed that the concentration producing a 100-fold decrease in virus titer of DENV-1 was 37 mg L^{-1}.

7.25 *Lippia alba and Lippia citriodora*

Lippia alba and *Lippia citriodora* belong to family Verbenaceae. They are flowering plants native to Southern Texas, Mexico, the Caribbean, Central and South America. Essential oils from *Lippia alba* and *Lippia citriodora* showed a considerable inhibitory effect

on dengue virus serotype replication in Vero cells[35]. A 50 % reduction in virus plaque number values was found with *L. alba* oil at between 0.4–32.6 lg mL^{-1} whereas for *L. citriodora* oil, the IC50 values were between 1.9 and 33.7 µg mL^{-1}. *L. alba* essential oil was more effective against DENV-2 than other serotypes, while for *L. citriodora* essential oil, the virucidal action against DENV-1, 2 and 3 were similar but lower than against DENV-4. Essential oil of *L. alba* was observed to produce a 100 % reduction of YFV yield at 100 µg mL^{-1} [77].

7.26 *Meristiella gelidium*

Meristiella gelidium belongs to family Solieriaceae. It is a marine species found in Atlantic Islands, North America, Caribbean Islands and South America. The antiviral activity of kappa carragenan (5) in *Meristiella gelidium* was evaluated against DENV-2[105]. The IC50 of carragenans isolated from *M. gelidium* was in the range of 0.14–1.6 µg mL^{-1}. The results show that the extract and the fraction derived from *M. gelidium* were more effective inhibitors of DENV-2 when compared with reference polysaccharides (heparin and DS 8000).

7.27 *Mimosa scabrella*

Mimosa scabrella belongs to family Fabaceae. It is a fastgrowing, 15–20 m high and up to 50 cm diameter tree native to the cool, subtropical plateaus of South eastern Brazil. Galactomannans[7] extracted from seeds of *Mimosa scabrella* have demonstrated activity against YFV and DENV-1 in vitro and in vivo[12]. M. scabrella showed protection against death in 87.7 % of YFV-infected mice. In vitro experiments with DENV-1 in C6/36 cell culture assays showed that a concentration of 347 mg L^{-1} produced a 100-fold decrease in virus titer of DENV-1.

7.28 Momordica charantia

Momordica charantia belongs to family Cucurbitaceae. It is also known as bitter melon or peria (Malaysia), a tropical and subtropical vine found throughout Asia, Africa and the Caribbean. The MNTD of the methanolic extract of *Momordica charantia* against Vero E6 cells was investigated in vitro[7]. M. charantia recorded a maximal dose that was not toxic to cells of 0.20 mg mL^{-1}. The methanolic extract of *M. charantia* showed inhibitory effect on DENV-1 by antiviral assay based on cytopathic effects.

7.29 *Ocimum sanctum*

Ocimum sanctum belongs to family Labiatae. It is an aromatic herb and shrub native to the tropical regions of Asia and the Americas. Tea, which is traditionally prepared by using *Ocimum sanctum* boiled leaves, acts as a preventive medicament against DF [106]. The MNTD of methanolic extract of O. sanctum against Vero E6 cells in vitro was investigated[7]. However, no significant difference in MNTD for O. sanctum was recorded. The methanolic extract of O. sanctum showed a slight inhibitory effect on DENV-1 based on cytopathic effects.

7.30 *Phyllanthus urinaria*

Phyllanthus urinaria is commonly called chmberbitter, gripeweed belongs to the family of Phyllanthaceae. It is believed that the plant originated in tropical asia and widely distributed in South India, South America and China. It is used for treatment of several diseases like Hepatitis, jaundice, Urinary Tract Infections, Syphillis, Asthma, Bronchitis, Anemia and joint pains etc. It was also found to have anti-cancer activity.7'-hydroxy-3',4',5,9,9'-pentamethoxy-3,4-methylene dioxy lignin isolated from the ethylacetate extract of P. urinaria was shown to exhibit anticancer activity[17] by inducing apoptosis. Recently this plant also shown to have anti dengue activity. Sau Har Lee et al., (2013)[107] has studied the anti-dengue effect of aqueous and methanolic extract of four species of Phyllanthus such as P.amarus, P.niruri, P.urinaria, P.wastonii. These species showed strongest inhibitory activity against DENV2 with more than 90% of virus reduction in simultaneous treatment at maximal non toxic dose of 250.0 µg/mL and 15.63 µg/mL

7.31 *Piper retrofractum*

Piper retrofractum belongs to family Piperaceae. It is a flowering vine native to Southeast Asia and cultivated in Indonesia and Thailand mostly for its fruit. In vitro anti-dengue activity of *Piper retrofractum* in Vero cells was investigated[2]. The inhibitory activity against DENV-2 infected cells was determined on dichloromethane ethanol extract by the MTT method. The ethanol extract of P. retrofractum exhibited an inactivated viral particle activity or 84.93 % at a concentration of 100 µg mL^{-1}. Previous study has shown that an aqueous extract of long pepper, *P. retrofractum*, gives the highest level of activity against mosquito larvae[108].

7.32 *Piper sarmentosum*

Piper sarmentosum belongs to the Piperaceae family which is economically important because of their medicinal and culinary uses. It is also called as Lolot Pepper and leaves of this plant are traditionally used as condiment and also used for its carminative property. The whole plant having medicinal properties and is used to treat inflammation, skin diseases, rheumatism, diarrhea and root is used for the treatment of cough and asthma. *Piper sarmentosum* contains many chemical constituents such as ascaricin, α-ascarone, β- sitosterols and also contains Vitamin C, Vitamin E, Carotenes, Xanthophylls etc. The ethanol extract of Piper sarmentosum possesses larvicidal effect against early 4^{th} instar larvae of Aedes aegypti mosquitoes. Udom et al., (2005)[109] has studied the larvicidal activity of three species of pepper plants on aedes aegypti.

7.33 *Psidium guajava*

Psidium guajava belongs to family Myrtaceae. It is an evergreen shrub or small tree indigenous to Mexico, the Caribbean and Central and South America. It is cultivated widely in tropical and subtropical regions around the world. Psidium guajava leaf extract has been tested in vitro and showed to inhibit the growth of dengue virus[110]. Water boiled with guava leaves was used to avoid bleeding in DHF, and increased platelet counts to 100.000/mm^3 within a period of approximately 16 h [83]. *P. guajava* ripe fruit or juice has healing properties in cases of DF by improving the declining levels of platelets [82].

7.34 *Quercus lusitanica*

Quercus lusitanica belongs to family Fagaceae. It is a species of oak native to Morocco, Portugal and Spain. *Quercus lusitanica* extract was found to have a good inhibitory effect on the replication of DENV-2 in C6/36 cells[72]. The methanol extract of the seeds completely inhibited (10–1,000 fold) the TCID50 of virus at its maximum non-toxic concentration of 0.25 mg mL^{-1} as indicated by the absence of cytopathic effects. A low dose of Q. lusitanica (0.032 mg mL^{-1}) showed 100 % inhibition with 10 TCID50 of virus. Proteomics techniqueswere used to demonstrate that the effect of Q. lusitanica was to down regulate NS1 protein expression in infected C6/36 cells after treatment with the extract.

7.35 *Rhizophora apiculata*

Rhizophora apiculata belongs to family Rhizophoraceae. It is a mangrove tree up to 20 m tall that grows in Australia (Queensland and Northern Territory), Guam, India, Indonesia, Malaysia, Micronesia, New Caledonia, Papua New Guinea, the Philippines, Singapore, the Solomon Islands, Sri Lanka, Taiwan, Maldives, Thailand and Vietnam. Anti-dengue properties of the ethanolic extract of *Rhizophora apiculata* in DENV-2 in Vero cells have been reported[2]. R. apiculata exhibited inhibitory activity and an inactivated viral particle activity of 56.14 % and 41.5 % at concentrations of 12.5 and 100 µg mL^{-1}, respectively.

7.36 *Tephrosia crassifolia, Tephrosia madrensis and Tephrosia viridiflora*

Tephrosia crassifolia, Tephrosia madrensis and *Tephrosia viridiflora* belong to family Fabaceae. Genus Tephrosia is an herb, undershrub or shrub, distributed mainly in tropical and subtropical regions of the world. Three species from this family (*Tephrosia crassifolia, Tephrosia madrensis* and *Tephrosia viridiflora*) were investigated[10]. The flavonoids isolated from *T. madrensis*, glabranine[8] and 7-O-methyl-glabranine[9] exert strong inhibitory effects on dengue virus replication in LLC-MK2 cells. Methyl-hildgardtol A isolated from *T. crassifolia* exhibited a moderate to low inhibitory effect, while hildgargtol A from T. crassifolia and elongatine from *T. viridiflora* had no effect on viral growth. Uncaria tomentosa Uncaria tomentosa belongs to family Rubiaceae. It is a woody vine growing in the tropical jungles of Central and South America. *Uncaria tomentosa* is a large wood vine native to the Amazon and Central American rainforests[111]. It is used widely as traditional medicine by native people of the Peruvian rainforest[112]. The antiviral activity of *U. tomentosa* was revealed by viral antigen

(DENV-Ag) detection in monocytes by flow cytometry in C6/36 cells[112]. The most effective activity emerged from the alkaloidal fraction of U. tomentosa. The pentacyclic oxindole alkaloid-enriched fraction of U. tomentosa was observed as most effective at decreasing DENV-Ag detection in monocytes at concentrations of 1 µg mL^{-1}, whereas the crude hydroethanolic extract demonstrates inhibitory activity at concentrations of 10 µg mL^{-1}.

7.37 *Zostera marina*

Zostera marina belongs to family Zosteraceae. It is an aquatic plant known as eelgrass and is native to North America and Eurasia. A compound from the temperate marine eelgrasss *Zostera marina* has been identified as possessing anti dengue virus activity in a focus-forming unit assay in LLCMK2 cells[74]. The anti-adhesive compound p-sulfoxycinnamic acid, zosteric acid, ZA[10], derived from Z. marina showed a modest IC50 of approximately 2.3 mm against DENV-2. The other compound with related chemistries, CF 238, showed the most activity, with IC50 values of 24, 46, 14 and 47 µM against DENV-1, DENV-2, DENV-3 and DENV-4, respectively.

7.38 *Uncaria tomentosa*

Cat's claw is a large, woody vine found in tropical South and Central America, including Peru, Colombia, Ecuador, Guyana, Trinidad and Venezuela. It is belongs to Rutaceae family having different medicinal properties. In addition to its immune stimulating activity, other in vitro anticancerous properties have been documented for these alkaloids and other constituents in cat's claw. Five of the oxindole alkaloids have been clinically documented with in vitro antileukemic properties, and various root and bark extracts have demonstrated. Anti-Viral activity of hydro-alcoholic extract of *Uncaria tomentosa* plant was evaluated on human monocytes infected with Dengue type 2 virus by Valente et al.,(2008)[113]. The results of this study demonstrated an invitro inhibitory activity by extracts reducing Dengue –Ag+ cell rates in treated monocytes.

8. CONCLUSION AND FUTURE DIRECTIONS

The development of new anti-dengue products from bioactive compounds is necessary in order to find more effective and less toxic anti-dengue drugs. Therefore, any extensive study on the potential of plants with isolated active compounds that have shown anti-dengue activity should go through additional in vitro and in vivo animal testing followed by toxicity and clinical tests. This route may reveal a promising compound to be optimized and thus be suitable for application in the production of new anti-dengue compounds. If pursued from drugs derived from medicinal plants around the continents, this work may prove valuable to the health of individuals and to nations. Moreover, such discoveries may lead to the development of highly efficient and safe anti-dengue treatments. However, to identify potential anti-dengue plants or compounds, knowledge of the mechanisms of virus infection need to be understood in order to facilitate the search for and development of the most appropriate drugs. Further research is needed to determine how to target the most appropriate stages to prevent the spread of virus infection. Focusing on each phase in the life cycle of the virus, new compounds could prevent (1) infection of host cells, (2) the viral maturation process, (3) synthesis of viral RNA, or (4) the spread of viral particles.

REFERENCES

1. Talarico LB, Zibetti RGM, Noseda MD, Duarte MER, Damonte EB, et al. "An algal-derived DL-galactan hybrid is an efficient preventing agaent for in vitro Dengue virus infection", *Planta Med*, 2007, 73:1464–1468.
2. Klawikkan N, Nukoolkarn V, Jirakanjanakir N, Yoksan S, Wiwat C, Thirapanmethee K, et al. "Effect of Thai medicinal plant extracts against Dengue virus in vitro", *MU J Pharm*, 2011, 38(1–2): 13–18.
3. Guzman A, Isturiz RE. "Update on the global spread of dengue", *Int J Antimicrob Agents*, 2010, 36S:S40–S42.
4. WHO. World Health Organization. "Dengue and severe dengue". Fact Sheet, 2012. http://www.who.int/mediacentre/factsheets/fs 117/en/
5. Leardkamolkarn V, Srigulpanit W, Phurimsak C, Kumkate S, Himakoun L, Sripanidkulchai B, et al."The inhibitory actions of Houttuynia cordata aqueous extract on Dengue virus and Dengue-infected cells" *J Food Biochem*, 2012, 26:86–92. doi:10.1111/j. 1745-4514.2010.00514.x.
6. Goel A, Patel DN, Lakhani KK, Agarwal SB, Agarwal A, Singla S, Agarwal R, et al. "Dengue fever—a dangerous foe", *J Indian Acad Clin Med,* 2004*,* 5(3):247–258.
7. Tang LIC, Ling APK, Koh RY, Chye SM, Voon KGL, et al. "Screening of anti-dengue activity in methanolic extracts of medicinal plants", *BMC Complement Altern Med*, 2012, 12:3.
8. Parida MM, Upadhyay C, Pandya G, Jana AM, et al. "Inhibitory potential of neem (Azidarachta indica Juss) leaves on Dengue virus type-2 replication",*J Ethnopharmacol*, 2002, 79:273–278.
9. Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, Fazal L, et al. "Dengue fever treatment with Carica papaya leaves extracts", *Asian Pac J Trop Biomed*, 2011, 1:330–333. doi:10.1016/ S2221-1691(11)60055-5.
10. Sanchez I, Garibay FG, Taboada J, Ruiz BH, et al. "Antiviral effect of flavonoids on the Dengue virus", *Phytother Res*, 2000, 14:89–92.
11. Jain M, Ganju L, Katiyal A, Padwad Y, Mishra KP, Chanda S, Karan D, Yogendra KMS, Sawhney RC, et al. "Effect of Hippophae rhamnoides leaf extracts against Dengue virus infection in human blood-derived macrophages" *Phytomedicine*, 2008, 15:793–799.
12. Ono L, Wollinger W, Rocco IM, Coimbra TLM, Gorin PAJ, Sierakowski MR, et al. "In vitro and in vivo antiviral properties of sulfated galactomannans against yellow fever virus (BeH111 strain) and dengue 1 virus (Hawaii strain)", *Antivir Res*, 2003, 60:201–208.
13. Gubler JD. "Dengue/dengue haemorrhagic fever: history and current status. In: Bock G, Goode J (eds) New treatment strategies for dengue and other flaviviral diseases", Wiley, Chichester (Novartis Foundation Symposium 277), 2006, pp 3–22.
14. Kimura R, Hotta S. "Studies on dengue fever (IV) on inoculation of dengue virus into mice" *Nippon Igaku,* 1944, 3379: 629-33.
15. Sabin AB, Schlesinger MC. "Production of immunity to dengue with virus modified by propagation in mice" *Science,* 1945, 101: 640-2.
16. Sarkar JK, Chatterjee SN, Chakravarty SK, et al. "Haemorrhagic fever in Calcutta: some epidemiological observations", *Indian J Med Res,* 1964, 52: 651-9.
17. Chatterjee SN, Chakravarti SK, Mitra AC, Sarkar JK, et al."Virological investigation of cases with neurological complications during the outbreak of haemorrhagic fever in Calcutta", *J Indian Med Assoc,* 1965, 45: 314-6.
18. Carey DE, Myers RM, Reuben R, Rodrigues FM, et al. "Studies on dengue in Vellore, South India", *Am J Trop Med Hyg,* 1966, 15: 580-7.
19. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV, et al. "Dengue and dengue hemorrhagic fever", *Lancet,* 1998, 352: 971-7.
20. Kabra S. K, Verma IC, Arora NK, Jain Y, Kalra V, et al. "Dengue haemorrhagic fever in children in Delhi", *Bull World Health Organ,* 1992, 70: 105-8.
21. Bhattacharjee N, Mukherjee KK, Chakravarti SK, Mukherjee MK, De PN, Sengupta M, et al. "Dengue haemorrhagic fever (DHF) outbreak in Calcutta – 1990", *J Commun Dis,* 1993, 25: 10-4.
22. Cherian T, Ponnuraj E, Kuruvilla T, Kirubakaran C, John TJ, Raghupathy P, et al. "An epidemic of dengue haemorrhagic fever & dengue shock syndrome in & around Vellore", *Indian J Med Res,* 1994, 100: 51-6.
23. Dar L, Broor S, Sengupta S, Xess I, Seth P, et al. "The first major outbreak of dengue hemorrhagic fever in Delhi, India.", *Emerg Infect Dis,* 1999, 5: 589-90.
24. Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, et al. "A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow", India", *Southeast Asian J Trop Med Public Health,* 1999, 30: 735-40.
25. Shah I, Deshpande GC, Tardeja PN, et al. "Outbreak of dengue in Mumbai and predictive markers for dengue shock syndrome", *J Trop Pediatr,* 2004, 50: 301-5.

26. Kyle JL, Harris E. “Global spread and persistence of dengue”, *Annu Rev Microbiol* , 2008, 62:71–92.
27. Qi RF, Zhang L, Chi CW, et al. “Biological characteristics of dengue virus and potential targets for drug design”, *Acta Biochim Biophys Sin*, 2008, 40(2):91–101.
28. Saleeza SNR, Rashid NY, Azirun MS, et al. “Mosquitoes larval breeding habitat in urban and suburban areas, Peninsular Malaysia”, *World Acad Sci Eng Technol*, 2011, 58:569–573.
29. Amarasighe A, Kuritsky JN, Letson GW, Margolis HS, et al. “Dengue virus infection in Africa”, *Emerg Infect Dis*, 2011, 17(8):1349–1354.
30. NaTHNaC. “Dengue Fever. Health Protection Agency”, Natural Travel Health Network and Centre, 2009.
31. CDC. “The Dengue Update: Dengue Outbreaks Worldwide”, Centre for Disease Control and Prevention, 2010, 2 (1.1).
32. Grzybowski A, Tiboni M, da Silva MAN, Chitolina RF, Passos M, Fontana JD, et al. “The combined action of phytolarvacides for the control of dengue fever vector, Aedes aegypti”, *Braz J Pharmacogn*, 2011, 22:549–557. ISSN 0102-695X
33. Leyssen P, Clercq ED, Neyts J, et al. “Perspectives for the treatment of infections with Flaviviridae”, *Clin Microbiol Rev*, 2000, 13(1):67–82.
34. Beaute J, Vong S. “Cost and disease burden of dengue in Cambodia”, *BMC Public Health*, 2010, 10:521–526.
35. Ocazionez RE, Meneses R, Torres FA, Stashenko E, eet al. “Virucidal activity of Colombian Lippia essential oils on Dengue virus replication in vitro”, *Mem Inst Oswaldo Cruz,* 2010, 105(3):304–309.
36. He JF, Luo HM, Liang WJ, Kui Z, Min K, Liu LP, et al.“Epidemic situation of dengue fever in Guangdong province, China, 1990–2005”, *Dengue Bull*, 2007, 31:1–9.
37. Veeraseatakul P, Wongchompoo B, Thichak S, Yananto Y, Waneesorn J, Chutipongvivate S, et al. “Circulation of dengue serotypes in five provinces of northern Thailand during 2002–2006”, *Dengue Bull*, 2007, 31:19–25.
38. Singh B, Goswami A, Chawla N, Shyam S, et al. “Role of helplines for dissemination of information during an outbreak of dengue fever in Delhi, India, in 2006: an experience”, *Dengue Bull*, 2007, 31:178–181.
39. Karamchandani PV. “Dengue group of fevers in India”, *Lancet,* 1946, 1: 92.
40. Carey DE, Myers RM, Reuben R, Rodrigues FM, et al. “Studies on dengue in Vellore, South India”, *Am J Trop Med Hyg,* 1966, 15: 580-7.
41. Ramakrishanan SP, Gelfand HM, Bose PN, Sehgal PN, Mukharjee RN, et al. “The epidemic of acute haemorrhagic fever, Calcutta, 163: epidemiological inquiry”, *Indian J Med Res,* 1964, 52: 633-50.
42. Sarkar JK, Pavri KM, Chatterjee SN, Chakravarty SK, Aanderson CR, et al. “Virological and serological studies of cases of haemorrhagic fever in Calcutta”, *Indian J Med Res,* 1964, 52: 684-91.
43. Chaudhuri RN, Saha TK, Chaudhuri AD, et al. “Dengue-like fever in Calcutta: further preliminary observations”, *Bull Calcutta Sch Trop Med,* 1965, 13: 2-3.
44. Krishnamurthy K, Kasturi TE, Chittipantulu G, et al. “Clinical and pathological studies of an outbreak of dengue-like illness in Visakhapatnam”, *Indian J Med Res,* 1965, 53: 800-12.
45. Paul SD, Dandawate CN, Banerjee K, Krishnamurthy K, et al. “Virological and serological studies on an outbreak of dengue-like illness in Visakhapatnam, Andhra Pradesh”, *Indian J Med Res,* 1965, 53: 777-89.
46. Balaya S, Paul SD, D’Lima LV, Pavri KM, et al. “Investigations on an outbreak of dengue in Delhi in 1967”, *Indian J Med Res,* 1969, 57: 767-74.
47. Chaturvedi UC, Mathur A, Kapoor AK, Mehrotra NK, Mehrotra RML, et al. “Virological study of an epidemic of febrile illness with haemorrhagic manifestations at Kanpur, India, during 1968”, *Bull World Health Organ,* 1970, 43: 289-93.
48. Chaturvedi UC, Kapoor AK, Mathur A, Chandra D, Khan AM, Mehrotra RML, et al. “A clinical and epidemiological study of an epidemic of febrile illness with haemorrhagic manifestations which occurred at Kanpur, India in 1968”, *Bull World Health Organ,* 1970, 43: 281-7.
49. Myers RM, Carey DE, Banerjee K, Reuben R, Ramamurti DV, et al. “Recovery of dengue type 3 virus from human serum and *Aedes aegypti* in South India”, *Indian J Med Res,* 1968, 56: 781-7.
50. Ghosh BN. “A study on the epidemic of dengue-like fever in Pondicherry (1964-65 and 1965-66)”, *J Indian Med Assoc,* 1968, 51: 261-4.
51. Chaturvedi UC, Mathur A, Kapoor AK, Tandon HO, Mehrotra RML, et al. “Clinicovirological study of the recurrence of dengue epidemic with haemorrhagic manifestation at Kanpur, during 1969”, *Indian J Med Res,* 1972, 60: 329-33.
52. Chaturvedi UC, Mathur A, Mehrotra RM, et al. “Experimentally produced cardiac injury following dengue virus infection”, *Indian J Pathol Bacteriol,* 1974, 17: 218-20.
53. Myers RM, Carey DE, De Ranitz CM, Reuben R, Bennet B, et al. “Virological investigations of the 1966 outbreak of Dengue type 3 in Vellore, Southern India”, *Indian J Med Res,* 1969, 57: 1392-401.
54. Myers RM, Varkey MJ, Reuben R, Jesudass ES, et al. “Dengue outbreak in Vellore, southern India, in 1968, with isolation of four dengue types from man and mosquitoes”, *Indian J Med Res,* 1970, 58: 24-30.
55. Myers RM, Varkey MJ. “A note on sequential dengue infection, presumptive and proved, with report of an instance of a third proved attack in one individual”, *Indian J Med Res,* 1971, 59: 1231-6.
56. Mahadev PV, Kollali VV, Rawal ML, Pujara PK, Shaikh BH, Ilkal MA, et al. “Dengue in Gujarat state, India during 1988 & 1989”, *Indian J Med Res,* 1993, 97: 135-44.
57. Ghosh SN, Pavri KM, Singh KR, Sheikh BH, D’lima LV, Mahadev PV, et al. “Investigations on the outbreak of dengue fever in Ajmer City, Rajasthan State in 1969, Part I. Epidemiological, clinical and virological study of the epidemic”, *Indian J Med Res,* 1974, 62: 511-22.
58. Chouhan GS, Rodrigues FM, Shaikh BH, Ilkal MA, Khangaro SS, Mathur KN, et al. “Clinical & virological study of dengue fever outbreak in Jalore city, Rajasthan 1985”, *Indian J Med Res,* 1990, 91: 414-8.
59. Rodrigues FM, Pavri KM, Dandawate CN, Banerjee K, Bhatt PN, et al. “An investigation of the aetiology of the 1966 outbreak of febrile illness in Jabalpur, Madhya Pradesh”, *Indian J Med Res,* 1973, 61: 1462-70.
60. Kumar A, Sharma SK, Padbidri VS, Thakare JP, Jain DC, Datta KK, et al. “An outbreak of dengue fever in rural areas of northern India”, *J Commun Dis,* 2001, 33: 274-81.
61. Parida MM, Dash PK, Upadhyay C, Saxena P, Jana AM, et al. “Serological & virological investigation of an outbreak of dengue fever in Gwalior, India”, *Indian J Med Res,* 2002, 116: 248-54.
62. Kurukumbi M, Wali JP, Broor S, Aggarwal P, Seth P, Handa R, et al. “Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi”, *Indian J Med Sci,* 2001, 55: 149-56.
63. Singh UB, Maitra A, Broor S, Rai A, Pasha ST, Seth P, et al. “Partial nucleotide sequencing and molecular evolution of epidemic causing dengue 2 strains”, *J Infect Dis,* 1999, 180: 959-65.
64. Dash PK, Parida MM, Saxena P, Kumar M, Rai A, Pasha ST, et al. “Emergence and continued circulation of dengue-2 (genotype IV) virus strains in northern India”, *J Med Virol,* 2004, 74: 314-22.
65. Anoop M, Issac A, Mathew T, Philip S, Kareem NA, Unnikrishnan R, et al. “Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India”, *Indian J Exp Biol,* 2010, 48: 849-57.
66. Mukherjee KK, Chakravarti SK, Dey PN, Dey S, Chakraborty MS, et al. “Outbreak of febrile illness due to dengue virus type 3 in Calcutta during 1983”, *Trans R Soc Trop Med Hyg,* 1987, 81: 1008-10.
67. Dash PK, Saxena P, Abhyankar A, Bhargava R, Jana AM, et al. “Emergence of dengue virus type-3 in northern India”, *Southeast Asian J Trop Med Public Health,* 2005, 36: 370-7.

68. Dash PK, Saxena P, Abhyankar A, Bhargava R, Jana AM, et al. “Reemergence of dengue virus type-3 (subtype-III) in India: implications for increased incidence of DHF & DSS”, *Virol J,* 2006, 3: 55-65.
69. Paramasivan R, Thenmozhi V, Thangaratham PS, Rajendran R, Tewari SC, Dhananjeyan KJ, et al. “An outbreak of dengue fever in Tirupur, Coimbatore district, Tamil Nadu”, *Indian J Med Res,* 2010, 132: 105-7.
70. Pang T, Hassan H, Ramalingam S, et al. “Demam denggi dan demam denggi berdarah”, *Dewan Bahasa dan Pustaka*, Kuala Lumpur, 1988.
71. Suaya JA, Shephard DS, Siqueira JB, Martelli CT, Lum LCS,Tan LH, Kongsin S, Jiamton S, Garrido F, Montoya R, Armien B, Huy R, Castillo L, Caram M, Sah BK, Sughayyar R, Tyo KR, Halstead SB, et al. “Cost of dengue cases in eight countries in the Americas and Asia: a prospective study”, *Am J Trop Med Hyg,* 2009, 80(5):846–855.
72. Muliawan SY, Kit LS, Devi S, Hashim O, Yusof R, et al. “Inhibitory potential of Quercus lusitanica extract on Dengue virus type 2 replication”, *Southeast Asian J Trop Med Public Health*, 2006, 37(9):132–135.
73. Muhamad M, Kee LY, Rahman NA, Yusof R, et al. “Antiviral actions of flavonoid-derived compounds on Dengue virus type-2”, *Int J Biol Sci,* 2010, 6(3):294–302.
74. Rees CR, Costin JM, Fink RC, McMichael M, Fontaine KA, Isern S, Michael SF, et al. “*In-vitro* inhibition of dengue virus entry by p-sulfoxy-cinnamic acid and structurally related combinatorial chemistries”, *Antivir Res,* 2008, 80:135–142.
75. Solomon T, Mallewa M. “Dengue and other emerging Flavi viruses”, *J Infect Dis*, 2001, 42:104–115.
76. WHO, World Health Organization, “Traditional medicine”, Fact Sheet, 2008. http://www.who.int/mediacentre/factsheets/fs134/en/
77. Meneses R, Ocazionez RE, Martinez JR, Stashenko EE, et al. “Inhibitory effect of essential oils obtained from plants grown in Colombia on yellow fever virus replication in vitro”, *Ann Clin Microbiol Antimicrob,* 2009, 8:8.
78. Betancur-Galvis LA, Saez J, Granados H, Slazar A, Ossa JE, et al. “Antitumor and antiviral activity of Colombian medicinal plant extracts”, *Mem Inst Oswaldo Cruz*, 1999, 94(4):531–535.
79. Kudi AC, Myint SH. “Antiviral activity of some Nigerian medicinal plants extracts”, *J Ethnopharmacol,* 1999, 68:289–294.
80. Philippine Medicinal Plants “Gatas-gatas”, 2011. http://www.stuartxchange.org/GatasGatas.html
81. The Cure Library. “Dengue fever cure using tawa–tawa aka gatas–gatas weed”, 2007. http://www.curelibrary.com/blog/2007/04.
82. About Health. “Dengue fever medicine”,2011.Available from URL: http://abouthealt-h.com/dengue-fever-medicine.
83. Healthy Lifestyle. “Guava leaf, prevent Dengue Haemorrhage”, 2010. http://www.secondlifeblogs.info/guava-leaf-prevent-denguehaemorrhage.html
84. Jiang WL, Luo XL, Kuang SJ, et al. “Effects of Alternanthera philoxeroides Griseb against dengue virus in vitro”, *J First Mil Med Univ*, 2005, 25(4):454–456.
85. CRC Weed Management. “Weed Management Guide—Alligator Weed”, (Alternanthera philoxeroides), 2003. http://www.weedscrc.org.au/documents/wmg_alligator_weed.pdf>.
86. Jiang, WL, Luo XL, Kuang SJ, et al. “Effects of Alternanthera philoxeroides Grisebagainst dengue virus in vitro”, *Di Yi Jun Yi Da Xue Xue Bao*, 2005, 25(4): 454 - 6.
87. Kanokwan J, Nobuo N. “Pharmacological Aspects of Andrographis paniculata on Health and Its Major Diterpenoid Constituent Andrographolide”, *Journal of Health Science*, 2008, 54 (4):370–381.
88. Mishra SK, Sangwan NS, Sangwan RS, et al. “Andrographis paniculata (Kalmegh): A re- view”, *Pharmacog. Rev*, 2007, 1:283–298.
89. Leon ICT, Anna PKL, Rhun YK, et al. “Screening of anti-dengue activity in methanolic extracts of medicinal plants”, *BMC Complementary and Alternative Medicine*, 2012, 12(3):1-10.
90. Kiat TS, Pippen R, Yusof R, Ibrahim H, Khalid N, Rahman NA, et al. “Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, Boesenbergia rotunda (L.), towards dengue-2 virus NS3 protease”, *BioOrg Med Chem Lett*, 2006, 16: 3337–3340
91. AR Mahesh, Harish Kumar, Ranganath MK, et al. “Detail Study on Boerhaavia Diffusa Plant for its Medicinal Importance- A Review”, *Research Journal of Pharmaceutical Sciences*, 2012, 1(1):28-36.
92. Bharati P, Sinha R. “Study the effect of Tinospora cardifolia (wild) miers and Boerhaavia diffusia linn on dengue”, *International Journal of Ayurvedic & Herbal Medicine*, 2012, 2(3):574-577.
93. Kevin W, Theodore CP, Brian G, et al. “Castanospermine, a Potent Inhibitor of Dengue Virus Infection In Vitro and In Vivo”, *Journal of Virology*, 2005, 79(14):8698-8706.
94. Talarico LB, Damonte EB. “Interference in dengue virus adsorption and un-coating by carrageenans”, *J. Virology*, 2007, 363(2):473-85.
95. Singh AA, Duggal SB, Singh JC, et al. “An inside preview of Ethnopharmacology of Cissampelos pareira linn”, *International Journal of Biological Technology*, 2010, 1(1):114-120.
96. Bhatnagar PK, Katiyar CK. (RanbaxyLaboratories Limited, India; International Centre for Genetic Engineering and Biotechnology;Department of Biotechnology-2010) “Anti-dengue activity of Cissampelos pareira extracts”, Application: WO 2010-IB50299 20100123, 2010.
97. Hidari KIPJ, Takahashi N, Arihara M, Nagaoka M, Morita K, Suzuki T, et al. “Structure and anti-Dengue virus activity of sulfated polysaccharide from marine alga”, *Biochem Biophys Res Commun*, 2008, 376:91–95.
98. Talarico LB, Zibetti RGM, Noseda MD, Duarte MER, Damonte EB, Faria PCS, Pujol CA, et al. “The antiviral activity of sulphated polysaccharides against Dengue virus is dependenton virus serotype and host cells”, *Antivir Res*, 2005, 66:103–110.
99. Qiu H, Tang W, Tong X, Ding K et al. “Structure elucidation and sulfated derivatives preparation of two alpha-D-glucans from Gastrodia elata Bl. and their anti-dengue virus bioactivities”, *Carbohydr Res*, 2007, 342(15):2230-6.
100. Pujol CA, Estevez JM, Carlucci MJ, Ciancia M, Cerezo AS, Damonte EB, et al. “Novel DL-galactan hybrids from the red seaweed Gymnogongrus torulosus are potent inhibitors of herpes simplex virus and dengue virus”, *Antivir Chem Chemother*, 2002, 13(2): 83–89.
101. Chirag AP, Kalyani D, Jalaram HT, et al.“Remedial Prospective of Hippophae rhamnoides Linn. (Sea Buckthorn)”, *ISRN Pharmacol*. 2012: 436857.
102. Jain M, Ganju I, Katiyal A, et al.“Effect of Hippophae rhamnoides leaf extract against dengue virus infection in human blood-derived macrophages”, *Phytomedicine*, 2008, 15(10):793-9.
103. Phurimsak C, Leardkamolkarn V. “Screening for antiviral effect of thai herbs; *Kaempferia parviflora, ellipeiopsis cherrevensis* and *stemona tuberosa* against dengue virus type-2”, 31st congress on science and technology of Thailand, Suranaree university of technology, 2005.
104. Srivastava M, Kapoor VP. “Seed galactomannans: an overview”, *Chem Biodivers*, 2005, 2:295–317.
105. Tischer PC, Talarico LB, Noseda MD, Guimaraes SMPB, Damonte EB, Duarte MER, et al. “Chemical structure and antiviral activity of carragenans from Meristiella gelidium against herpes simplex and dengue virus”, *Carbohyd Polym*, 2006, 63:459–465.
106. Mohan L, Amberkar MV, Kumari M, et al. “Ocimum sanctum Linn (Tulsi)—an overview”, *Int J Pharm Sci Rev Res*, 2011, 7(1):51–53. (ISSN 0976-044X)
107. Sau HL, Yin QT, Anusyah R et al.”Effects of cocktail of four local malaysian medicinal plants (phyllanthus spp.) against dengue virus 2”, *BMC Complementary and Alternative Medicine*, 2013, 13:192.
108. Chansang U, Zahiri NS, Bansiddhi J, Boonruad T, Thongsrirak P, Mingmuang J, Benjapong N, Mulla MS, et al. “Mosquito larvacidal activity of aqueous extracts of long pepper (Piper retrofractum Vahl) from Thailand”, *J Vector Ecol*, 2005, 30(2):195–200.

109. Udom C, Wej C, Kittichai K, et al. “Larvicidal effect of pepper plants on aedes aegypti (1) (diptera: culicidae)”, *Journal of Vector Ecology*, 2006, 31(1):138-144.
110. Pink R.“Guavaleaf extract potential cure dengue fever”, 2011. http://pinkroses.info/guava-leaf-extract-potential-cure-dengue-fever
111. Reis SRIN, Valente LMM, Sampaio AL, Siani AC, Gandini M, Azeredo EL, D’Avila LA, Mazzei JL, Henriques MGM, Kubelka CF, et al.“Immunomodulating and antiviral activities of Uncaria tomentosa on human monocytes infected with Dengue Virus-2”, *Int Immunopharmacol*, 2008, 8:468–476.
112. Vijayan P, Ragu C, Ashok G, Dhanaraj SA, Surej B, et al. “Antiviral activity of medicinal plants of Nilgiris”, *Indian J Med Res*, 2004, 120:24–29.
113. Reis SRIN, Valente LMM, Sampaio AL, et al. “Immunomodulating and antiviral activities of Uncaria tomentosa on human monocytes infected with DenguVirus-2”, *International Immunopharmacol*, 2008, 8(3):468–476.