WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 262-291. Review Article ISSN 2277– 7105

ANTI-DENGUE PHYTOCHEMICALS FROM VALUABLE PLANTS: A REVIEW

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Article Received on
12 September 2017,
Revised on 03 Oct. 2017,
Accepted on 24 Oct. 2017
DOI: 10.20959/wjpr201714-9973

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ABSTRACT

Dengue is one of the most concerned problem for all over the world. Many reports were submitted of dengue fever from various countries. Due to its different serotypes, it became very difficult to analyses appropriate drug compound. Thus, there is need of potential drug compound to target dengue virus. This review article focuses on some of the potential extracts from *Psidium guajava, B. rotunda* and *Cucurbita pepo* as anti-dengue agents. Many experimental study had shown the possible effect of plants extracts as anti-dengue agents using different studies. Thus, these important and useful phytochemicals can be used further for detailed analysis to reveal a promising compound in the production of new anti-dengue compounds. Phytochemicals from medicinal plants were good and less toxic to the health of the people. Hence, concluded that such discoveries may lead to the development of highly efficient and nontoxic anti-dengue compounds from common valuable plant extracts.

KEYWORDS: Dengue, Phytochemicals, *Psidium guajava, B. rotunda and Cucurbita pepo.*

DENGUE

Viral diseases are responsible for significant morbidity and mortality universally. They are the major threat to public health across the world. Many cases of viral diseases have been reported from different regions of the world including India (Pushpa *et al.*,2013).

Dengue fever is mosquito-borne infectious disease in many tropical and sub-tropical countries. Dengue is caused by dengue virus (DENV), a mosquito-borne Flavivirus (Sherin, 2011). Dengue fever is transmitted by the bite of a female *Aedes aegypti* mosquito infected

with a dengue virus. The mosquito becomes infected, when it bites a person with dengue virus in their blood. It can't be spread directly from one person to another person (Singh and Sinha, 2015). Dengue is one of the most common arthropod-borne viral diseases in human and a leading cause of illness and death in the world. This disease called as a "break-bone" fever because it sometimes causes severe joint and muscle pain like bones are breaking. Many reports were noted about dengue fever for more than 200 years. It is thought to account for 400 million cases annually among which approximately 3.97 billion people are at risk of infection in 128 endemic countries (Kadir *et al.*, 2013).

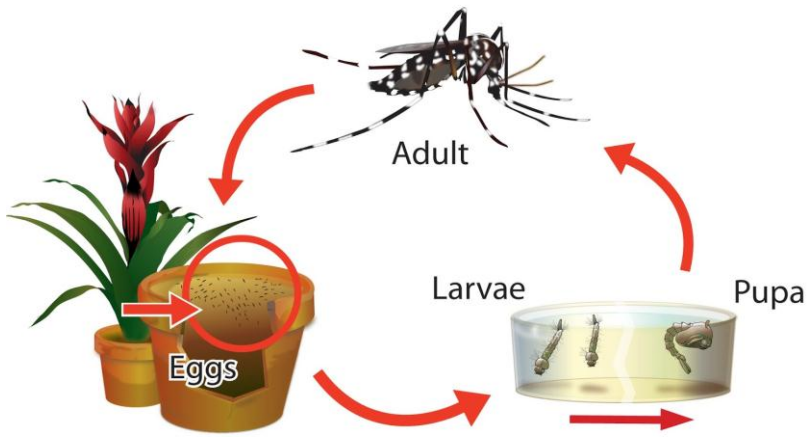

Fig. 1: Female *Aedes aegypti* mosquito life cycle.

The first clinical report of the dengue fever was in the 1789 by B. Rush, although the Chinese may have described the disease associated with "flying insects" before 420 AD. African described dengue as "ka dinga pepo" for the cramp-like seizure caused by an evil spirit. The Spanish may have changed the "dinga" to dengue since it means fastidious or careful in Spanish, which describes the gait of the people trying to reduce the pain of walking (N Powers and N Setzer, 2016).

Transmission

All four serotypes of dengue virus have a common history, including humans as the primary vertebrate host and *Aedes* mosquitoes of the subgenus *Stegomyia* like *Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis* as the primary mosquito vectors.

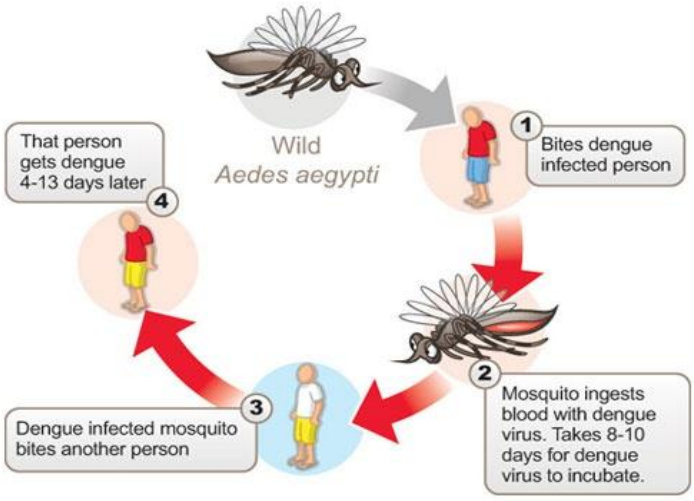

Fig. 2: Transmission of dengue virus.

It also exists in enzootic and epizootic forest cycles with non-human primates as the vertebrate host. Mostly, other vertebrate species are not susceptible to dengue viruses, other than neonatal mice challenged intracerebrally. The virus initially replicates in target organs like the liver, spleen, and thymus but eventually makes its way to the lymphatic system where it replicates in lymph tissue and white blood cells. The lymphatic system serves as a gateway to the bloodstream where the virus continues to replicate (Hacker, 2009). Transmission of dengue virus arises when an uninfected mosquito bites an infected host and takes up virus with its blood meal. After ingestion of a blood meal containing virus, there occur infection of the epithelial cells lining the midgut. The virus then outflows from the midgut epithelium into the haemocele and infects the salivary gland. Lastly, virus is secreted in the saliva, which cause infection during probing. The genital tract is also infected, and virus enter the fully developed egg at oviposition (Ayukekbong *et al.,* 2017).

For transmission, female *A. aegypti* must bite an infected human during the viraemic phase of the illness which usually lasts for 4 to 5 days and which last up to 12 days. *A. aegypti* may be infected with 2 different viruses without affecting the yield of either virus. The extrinsic incubation period is the time required for itself to become infective. This period is about 8 to 12 days. The feeding behaviour of the mosquito is considered as easily interrupted feeding and repeated probing of one or several hosts. Generally, *A. aegypti* has a low exposure to oral infection with dengue virus, thus it remains the most important vector because of its highly domesticated habits. Therefore, the determination of dengue virus depends on the development of high viral level in hosts to ensure transmission in mosquitoes. This vector-

virus relationship is the major factor in selecting and propagating pathogenic strains of dengue (Dhara *et al.*, 2016; Chawla *et al.*, 2014).

Signs and Symptoms

In endemic areas, most patients with dengue fever are either asymptomatic or present with mild febrile illness.

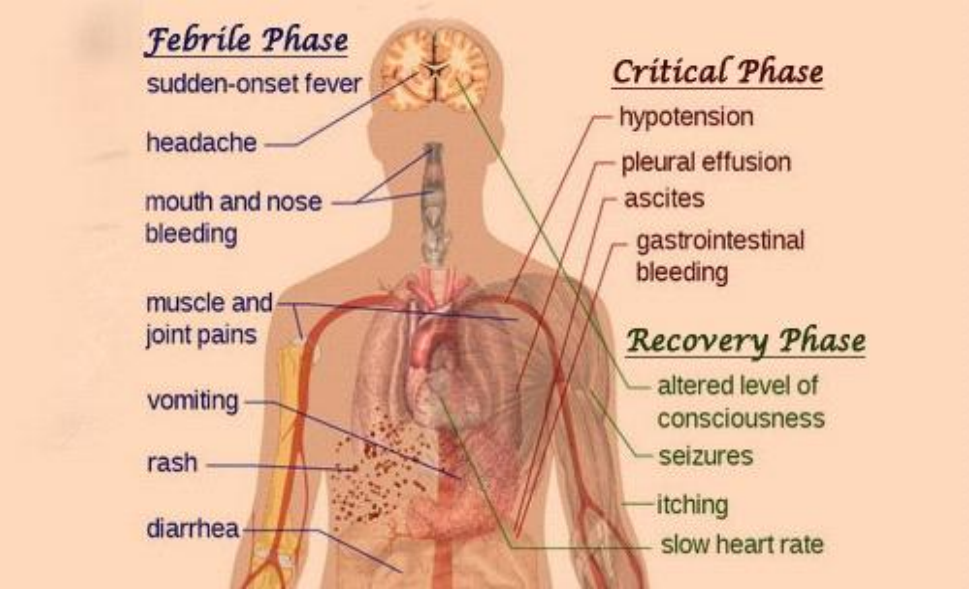

Fig. 3: Phases during dengue fever.

The infection ranges from asymptomatic infection, through undifferentiated fever and benign dengue fever to severe haemorrhagic fever with or without shock syndrome. It has more severe illness, and life-threatening. As incubation period ranges from 3-14 days, but mostly it is 4-7 days. Therefore, travellers returning from endemic areas are unlikely to have dengue if fever or other symptoms start from more than 14 days after arriving home. Children often experience symptoms like those of the common cold and gastroenteritis and generally have less severe symptoms than adults, but they are more prone to the severe complications (Chawla *et al.*, 2014; Sellahewa, 2012).

DENGUE VIRUS

Dengue virus (DENV) is a member of enveloped, positive-strand RNA viruses of the flaviviridae family. The flaviviridae family also includes West Nile virus, Yellow fever virus, Japanese encephalitis virus, Hepatitis C virus, and Tick-borne encephalitis virus. Flaviviruses are transmitted to humans by arthropod vectors such as mosquitoes (Mahadev *et al.*, 1993).

Dengue Virus Structure

Dengue infections are caused by four closely related viruses named DENV-1, DENV-2, DENV-3, and DENV-4. These four viruses are called serotypes because each has the different interactions with the antibodies in human blood serum. The four dengue viruses are almost similar as they share approximately 65% of their genomes but even within the single serotype, there is some genetic variation. Other than these variations, infection with each of the dengue serotypes results in the same disease with same range of clinical symptoms (Severson and Behura, 2012). As the infection with one serotype of dengue virus provides lifelong immunity, however, there is no immunity to infection of other serotype. All serotypes of dengue virus types may infect mainly a person living in an endemic area. The cycle of infection was maintained by viruses which use *A. aegypti* mosquito as a vector to infect the human host, in turn which serves as sources of viral amplification. The vector is a small domesticated, black and white tropical insect that feed on human during the daytime. There are two peaks of biting activity, one is in early morning for 2 to 3 hours and another in the afternoon for many hours before dark. It also breads in artificial containers in and around homes. Female *A. aegypti* feeds on several persons and may transmit dengue virus to them in short course of time (Anjum *et al.*, 2015).

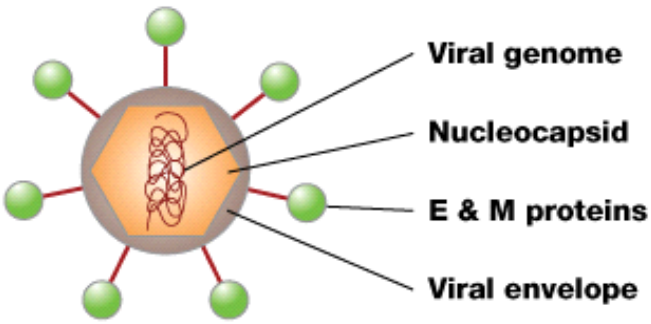

Fig. 4: Viral structure.

The virus has about 11000 bases genome that encodes a single large polyprotein that is subsequently cleaved into several structural and non-structural mature peptides (Hacker, 2009). The polyprotein divided into three structural proteins: C, prM, E and seven nonstructural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5 and short non-coding regions on both the 5' and 3' ends. These nonstructural proteins play roles in viral replication and assembly.

Structural Proteins

E protein

The DENV E (envelope) protein found on the viral surface, which is important for the primary attachment of the viral particle to the host cell. Various molecular interaction is occurred through viral E protein with ICAM 3-grabbing non-integrin, CD 209, Rab 5, GRP 78, and the mannose receptor comprise essential factors for attachment and viral entry (Singh and Sinha, 2015).

prM/M protein

The DENV prM (membrane) protein is important for formation and maturation of the viral particle including seven antiparallel β-strands maintained by three disulfide bonds (Mahadev *et al.*, 1993). The glycoprotein of the mature DENV virion contains 180 copies of the E protein and M protein. The pr peptide linked with the E protein till the viral particle is released into the extracellular environment. This pr peptide behaves like a cap, which covers the hydrophobic fusion loop of the E protein (Ayukekbong *et al.*, 2017).

Nonstructural Protein

NS1 protein

The first nonstructural protein 1 is about 48,000 molecular weight glycoproteins, hydrophilic, water-soluble and monomeric glycoprotein. NS1 may remain intracellular and transported to the plasma membrane. Secretion of large amounts of NS1 may be barred to infected mammalian, but not to mosquito cells (Sellahewa, 2012; Mahadev *et al.*, 1993).

NS2 protein

The NS2 coding region consists of two hydrophobic proteins, NS2a and NS2b of about 20,000 and 14,500 molecular weight, respectively. It is used for proteolytic processing of the C terminus of NS1.

NS3 protein

NS3 is the serine protease of 70,000 molecular weight and are hydrophilic protein. It is also known as RNA helicase and RTPase/NTPase. The protease domain consists of six β-strands arranged into two β-barrels formed by residues 1-180 of the protein. The catalytic triad contain His-51, Asp75 and Ser-135 between two β-barrels, and its activity is dependent on the presence of the NS2B cofactor. This cofactor wraps around the NS3 protease domain and turn into the part of the active site. The remaining NS3 residues form the three sub-domains

of the DENV helicase of a six-stranded parallel β-sheet bounded by four α-helices build sub-domains I and II, and III, which is composed of 4 α-helices surrounded by three shorter α-helices and two antiparallel β-strands (Sellahewa, 2012; Mahadev *et al.,* 1993; Severson and Behura, 2012; Anjum *et al.,* 2015; Gupta *et al.,* 2012).

NS4 protein

It is like proteins encoded in the NS2 region, having NS4a and NS4b of 16,000 and 27,000 molecular weight, respectively which are hydrophobic.

NS5 protein

The DENV NS5 protein is a 900-residue peptide with a methyltransferase domain at its N-terminal end from residues 1-296 and a RNA-dependent RNA polymerase (RdRp) at its C-terminal end from residues 320-900. The methyltransferase domain consists of an α/β/β sandwich flanked by N and C-terminal subdomains. The DENV RdRp is like other RdRps containing palm, finger, and thumb subdomains and a GDD motif for incorporating nucleotides (Gupta *et al.,* 2012).

As DENV serotype has been divided into four serotypes and it has been differentiated by target genome position in the sequence of 5' to 3' targeting 3' NCR of the viral genome such as (i) DENV-1 target genome position at 10469-10667 of 199 bp, (ii) DENV-2 at 10449-10659 of 211 bp, (iii) DENV-3 at 10289-10506 of 218 bp, (iv) DENV-4 at 10289-10517 of 229 bp.

Epidemiology of Dengue

Notable growth has been seen around the world in the percentage of dengue over the last two decades. Forty percent of the world's population living in areas at high risk for infection. The World Health Organization (WHO) estimates that there are between fifty and one-hundred million DENV infection cases each year, which cause hospitalization of five-hundred thousand people, and a death rate of two-and-a-half percent (Gupta *et al.,* 2012). Travelers from non-endemic places to the dengue affected places are straight forwardly exposed to the infection. Thus, this makes it a worldwide public health concern. The epidemiology of dengue fevers in the Indian subcontinent has been very intricate and shown significantly transformation over nearly past six decades in provisions of prevalent strains, which affected geographical areas and severity of disease (Mathew *et al.,* 20 16).

The earlier report of disease with dengue like symptoms correlates with the Chinese encyclopedia of disease symptoms and remedies that was used from 265 to 420 A.D during the Chin Dynasty. DENV as etiological agent was speculated during disease outbreaks in the French West Indies in 1635, in Panama in 1699, and the Philadelphia epidemic in 1780. Reported cases of dengue disease were seen in 1779 and 1780 in Africa, Asia, and North America (Halstead, 1988). The first confirmed dengue widespread arisen from 1953 to 1954 in the Philippines. at that time, this outbreak was believed to be among other hemorrhagic fevers, but confirmed to be dengue in 1958 through serological testing (Fusco and Chungm 2014). DENV spread and increased in the 1970's in Asia, the Pacific Islands, and the Americas. In the 1980's and 1990's, DENV continued to expand, and reached areas with mosquito vectors. Factors responsible for the increase in dengue epidemics are population growth and development, deterioration in water quality, suboptimal waste management, the lack of effective mosquito control, and human air and ship travel (Amat-ur-Rasool *et al.*, 2015).

The World Health Organization (WHO) has developed fixed criteria for diagnosing and classifying dengue disease. Dengue fever is confirmed, if the patient has an acute illness with two or more of the following symptoms such as headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, leucopenia and diagnosed serology by ELISA or illness concurrent with a confirmed dengue case (Frimayanti *et al.*, 2011). It can be also confirmed in a laboratory by one of the following way: virus isolation through cell culture or mosquito inoculation, viral antigen detection with ELISA, immunohistochemistry or immunofluorescence, a four-fold increase in antibody and viral RNA detection by PCR (Galiano *et al.*, 2016).

Currently Available Treatment Alternatives

As treatment is guided by etiology and disease severity study. The management of dengue includes symptomatic treatment with fluid management. Till now, no specific medicine is available (Mathew *et al.*, 2016). One of the important challenge is to estimate the severity of the disease as early as possible. Unlike most of the other diseases, a significant stage of the disease is the patient's defervescence during which increased vascular permeability may appear at the same time as viral enters and body temperature starts falling. Close detection of this, which may need intravascular fluid replacement and maintenance of good haemodynamic stability is important for disease result. Primary detection of disease and

classification will be the key in the prior implementation of an effective and appropriate antiviral therapy. The more knowledge about the host response to a dengue infection is the useful thing to cure it. Thus, knowledge of DENV components and life-cycle lead to efficient antiviral production through possibly in combination with those targeting cellular targets (Canard, 2012).

The study of pre-feeding mice with the trivalent chromium picolinate (CrP) in drinking water could stop the adverse effects of DV infection taking place for most of the hematological parameters. Through analysis, Similarly, acetaminophen can be used to treat patients with symptomatic fever. Theoretically, Aspirin, Brufen like nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics and corticosteroids should be avoided as these cause gastritis or bleeding (Mathew *et al.,* 2016; Fusco and Chungm 2014).

Molecular docking software, ZDOCK was used to perform docking, to predict the interactions between the dengue envelope and 2G12 antibody. This program results the 100 most probable predictions based on the electrostatic complementarity, hydrophobicity and the geometry of the molecular surface from thousands of candidates. Factors such as (i) predicted conserved epitopic residue in the interaction sites, (ii) residue conservation of the interaction sites, (iii) participation of the DC-MAN and antibody 2G12-MAN binding residue in the selected complexes were considered to select docked complexes. A unique solution was obtained through this three-step filtering method. Energy minimization was applied on the final solutions to obtain stable structures (Hassandarvish *et al.*,2016).

ETHNOBOTANY

Ethnobotany means the anthropological study of botany. In 1895, Harshberger coined this term to show plants used by the natives. It includes the study and evaluation of plant-human relationship in all phases and the effect of plant environment on human society. Later, Schultes in 1962 defined ethnobotany as the study of the relationship which exists between people of primitive societies and their plant environment. Plants have been used by tribal as well as local people to cure various diseases. As most of the diseases of modern society are called as life style disease and it can be cured by using herbal medicines. This includes all the plants used for food, medicine, divination, cosmetics, dyeing, textiles, for construction, tools, currency and clothing (Kumar *et al.,* 2015; Solanki *et al.,* 2017).

Potential Phytochemicals from Plant to Treat Dengue

Now-a-days medicinal plants have become a unique approach to safe, effective and inexpensive new remedies for various disease as they do not have adverse side-effects. It is assessed that 70-80% of people world widely dependent on traditional herbal medicine to fulfil their primary health care needs as well as for future generation and livelihood improvement. In the field of traditional medicines, India has a rich cultural heritage consists of two systems of treatment including Ayurvedic and Unani systems (Pushpa *et al.*,2013). According to a study in 1993 of WHO, the experts of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh. The Indian medicinal plants help to do successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite and in treating gastric, hepatic, cardiovascular & immunological disorders (N Powers and N Setzer, 2016). It is miserable that only few studies have been done to show that different plant extract can provide better antiviral outcomes compared to their synthetic analogues. Some of the plants which shows antiviral activity against dengue fever were listed as *Andrographis paniclata, Azadirachta indica, Boerhaavia diffuza, Chondrus crispus* and *Catanospernum austral* (Kumar *et al.*, 2015). The active compounds of plants belong to various chemical classes such as sulfated polysaccharides, flavonoids, quercetin and natural chalcone compounds (Kadir *et al.*, 2013; Shivendra *et al.*, 2012).

Also, plants are rich source of essential oils, glyceridic oils with mosquito repellent activities. Several plants derived mosquito repellent products have been patented which have been widely reviewed. The plant extract has been patented against all serotypes of dengue virus (Shivendra *et al.*, 2012). *Hippophae rhamnoides* leaf extract has been shown an important anti-dengue activity (Mathew *et al.*, 2016). The anti-dengue activity of baicalein and baicalin flavonoids against different stages of the virus replication cycle in the Vero cells had been studied. The study shown the possible interactions between the viral protease protein important for DENV replication with two flavonoids as potential candidates for the anti-dengue drug discovery with known *in-vitro* anti-dengue activity. Both compounds bind with viral proteins, as receptors, through hydrogen bonding and the other interactions such as pi–pi interactions, pi–sigma interactions and pi–cation interactions. The *in vitro* anti-protease assay showed the significant anti-dengue protease activity for both the compounds, particularly

baicalein, which can affect DENV intracellular replication and internalization than baicalin (Noble *et al.,* 2012; Toepak and Tambunan, 2017).

Carica papaya commonly called as papaya. It is a small, frost-tender, succulent, broadleaf evergreen tree which bears papaya fruits for the whole year. Tree has a single, unbranched, non-woody trunk bearing the scars of old leaf bases. It is commercially grown in southern Florida, southern California and Hawaii. Many compounds and an enzyme papain extracted from the milky sap of green fruit. It is used as medicine for Urinary troubles, Intestinal worms, Constipation, Fever, Indigestion, Dyspepsia, Wounds, Infections, Toothache, Parasitic worms, Arthritis as well as consuming papaya after a meal encourages proper digestion and prevents indigestion as well as bloating.

The papaya plant takes about its effect in dengue by treating the thrombocytopenia, which is associated with dengue (Senthilvel *et al.,* 2013). I*n vitro* studies found that the *C. papaya* L. leaf extracts inhibited heat-induced and hypo tonicity-induced hemolysis of the erythrocytes conquered from both the healthy persons as well as persons with dengue infection, in which the effect was observed with the lower concentrations of leaf extracts (Shah *et al.,* 2013). Thus, the extracts are expected to contain membrane-stabilizing properties and protection of blood cells against stress-induced destruction. This is may be due to the presence of the flavonoids and other phenolic compounds in the papaya leaves (Hacker, 2009).

The research conducted on 70 dengue fever patients, which concluded that papaya leaf juice helps to increase the white blood cells and platelets, also normalizes clotting, and repairs the liver (Yogiraj *et al.,* 2014).

A ready to serve beverage had been prepared including papaya leaves and guava against dengue fever. As papaya leaves contain various phytoconstituents like saponins, tannins, cardiac glycosides and alkaloids, which act on the bone marrow and prevent its destruction and boost its ability to produce platelets. Additionally, guava fruits are rich in vitamin C (ascorbic acid) content. It was concluded that the papaya leaves make the rapid increase in platelet count and immunity, which is the important factor for the management of dengue fever.

The increasing property of *Carica papaya* leaves juice was studied as an open labelled randomized controlled trial, which was carried on 228 patients with DF and dengue

haemorrhagic fever (DHF). Nearly, half the patients received the juice, for 3 consecutive days while the others remained as controls and established the standard management. The full blood count was monitored 8 hours for 48 hours. Gene expression studies were conducted on the ALOX 12 and PTAFR genes using ANCOVA. The result showed that mean platelet count in intervention group was significantly higher than control group after 40 or 48 hours of usage of juice. The ALOX 12 and PTAFR genes were highly expressed among those individuals on the juice, which concluded that *Carica papaya* juice shown significantly increase in the platelet count in patients with DF and DHF (Rahmani and Aldebasi, 2016). Increased platelets could lead to reduced bleeding, which help to avoid the progression to the severe illness of DHF (Kadir *et al.*, 2013).

Study of Important Plants to Treat Dengue

Psidium guajava

Kingdom - Plantae
Subkingdom - Tracheobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Rosidae
Order - Myrtales
Family - Myrtaceae
Genus - *Psidium*
Species - *Psidium guajava*

Fig. 5: *Psidium guajava* plant.

The common name of *Psidium guajava* is guava, yellow guava or lemon guava belongs to family Myrtaceae. It is widely cultivated in tropical and subtropical regions around the world. It is an evergreen shrub or small tree native to the Caribbean, Central America, India, South America, South Africa, Hawaii, Colombia, Puerto Rico, Jamaica, Brazil, and Israel. The genus *Psidium* contain about 133 genera and more than 3,800 species. It is the hardiest among all tropical fruit trees and give good outcomes in productivity and adaptability comparatively to other fruit crops. Guava tree shows a great diversity in tree size, bearing habit and yield. The presence of a huge number of rootstocks, cultivars and clones by vegetative propagation, leads to a great need of identifications of cultivars for nursery and growers (Chaudhary and Tripathi, 2014).

Uses

Generally, guava fruit is round, egg or pear shaped, which turns from green to yellowish in color as it matures. The various compounds from plant are used for medicine purpose for inflammation, diabetes, hypertension, caries, wounds, pain relief, fever, diarrhea, increase blood platelets, rheumatism, lung diseases, and ulcers etc. Guava fruit contains vitamin C, vitamin A, potassium and calcium. It is a good source of iron and pectin, also promotes digestion. It has been well studied that the vitamin C in guava is five times more than that of an orange. It is also used as an antiseptic, astringent and anthelmintic. As it kills bacteria, fungi and amoeba. The fresh leaves of the plant are very effective for toothaches as well as cough and throat pain. Also, it is used to cure epilepsy, cholera and convulsion in children.

The root, leave, bark and immature fruits of guava are used for gastroenteritis as they are astringents. Its fibre content controls blood pressure and cholesterol as it is very helpful for heart and help kidney in eliminating wastes. Largely, plant is used for phytotherapy around the world because of its various pharmacological activities. Traditionally, at many parts of the world the leaves and bark are used to eject placenta after childbirth (Shekins and Dorathy, 2014; Díaz-de-Cerio *et al.*, 2017).

The peel color of the ripen fruit is yellow, and the color of the flesh can be white, pink, yellow, salmon, or carmine, depending on the variety. The flesh contains numerous scleroids. Because of its numerous functions, there is a great interest in investigating good analytical data on carotenoids. Mainly epoxides and (Z)-isomers were analysed from high-performance liquid chromatography. In 1987, it was concluded that lutein found as the single carotenoid in the peel of white guava. Further, in 1986 it was analysed that it contains seven carotenoids.

Many study have insufficient information in the literature about the carotenoid composition of fruits based on chromatography and UV-visible spectra (Mercadante *et al.*, 1999).

It also includes a wide-range of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, proteins, sesquiterpenoid alcohols, triterpenoid acids, alkaloids, glycosides, steroids, flavanoids, tannins and saponins. Moreover, it is very rich in antioxidants and vitamins as well as in lutein, zeaxanthine and lycopene.

The leaves of guava are rich in quercetin. Guava's therapeutic activity is credited to quercetin. It is confirmed with antibacterial activity and immune-stimulatory agents. Quercetin is supposed to contribute to the anti-diarrhea as well as it can relax intestinal smooth muscle and inhibit bowel contractions. Other flavonoids and triterpenes in guava leaves show antispasmodic activity. Guava also have antioxidant properties because of the polyphenols found in the leaves. Main Guava's plant chemicals includes alanine, alpha-humulene, alpha-hydroxyursolic acid, alpha-linolenic acid, alpha-selinene, amritoside, araban, arabinose, arabopyranosides, arjunolic acid, aromadendrene, ascorbic acid, ascorbigen, asiatic acid, aspartic acid, avicularin, benzaldehyde, butanal, carotenoids, caryophyllene, catechol-tannins, crataegolic acid, catechins, D-galactose, D-galacturonic acid, ellagic acid, ethyl octanoate, ferulic acid, gallic acid, glutamic acid, goreishic acid, guafine, guavacoumaric acid, guaijavarin, guajiverine, guajivolic acid, guajavolide, guavenoic acid, guajavanoic acid, histidine, hyperin, ilelatifol D, isoneriucoumaric acid, isoquercetin, jacoumaric acid, kaempferol, lectins, leucocyanidins, limonene, linoleic acid, linolenic acid, lysine, mecocyanin, myricetin, myristic acid, nerolidiol, obtusinin, octanol, oleanolic acid, oleic acid, oxalic acid, palmitic acid, palmitoleic acid, pectin, polyphenols, psidiolic acid, quercetin, quercitrin, serine, sesquiguavene, terpenes, and ursolic acid. The guava fruits are either eaten fresh, or as drinks, ice cream, and preservers. Guava fruit is still used as a sweet treat by indigenous peoples throughout the rainforest (Joseph and Priya, 2011).

*In vit*ro study of *Psidium guajava* leaf extract showed the inhibition properties against the growth of dengue virus. As it helps to increases platelet counts in patients with the dengue fever. Boiled water with guava leaves was used to avoid bleeding in dengue haemorrhagic fever with increase in platelet counts to 100000/mm3 within a period of nearly 16 hours (Kadir *et al.*, 2013; Rojas-Garbanzo *et al.*, 2017).

Some studies also concluded that combination of angkak and ethanol extract of guava leaves could increase erythrocyte number and hematocrit value and used for the treatment for thrombocytopenia. The result of many studies showed notable molecular evidences of trombinol compound of guava as a potential drug candidate to treat diseases related to thrombocytopenia condition, including dengue fever, liver failure, coagulation disorder, and detrimental side effect of chemotherapy (Berlian *et al.*, 2017).

Much studies on kaempferol in guava has been done for pharmacological activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic. The combination of kaempferol and quercetin content of extracts stated a synergistic effect of antiproliferative in culture of Human cancer cell lines (Batubara *et al.*, 2017; Anand *et al.*, 2016).

Lycopene from guava used to reduces the risk of cancer. Also, it is associated with the prevention of cardiovascular damage due the LDL oxidation, as the impact of dyslipidemia. Further, it used to prevent or delay the oxidative damage of lipids, proteins, nucleic acids caused by reactive oxygen species, and in lowering lipid peroxidation as a contributing factor to atherosclerosis (Maryanto, 2013).

There is increase in demand for fruit beverages in many countries. As fruit beverages have nutritional, medicinal and calorie values compared to synthetic beverages. It can have excellent flavour, delicious taste and high nutritive and therapeutic values. Thus, the suitable combination of papaya leaves, and guava juice prepared to serve as beverage for the fast recovery of dengue fever with nutrients and good quality (Maryanto, 2013; Prabha and Thomas, 2014).

Boesenbergia rotunda

Kingdom - Plantae
Subkingdom - Tracheobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Liliopsida
Subclass - Zingiberidae
Order - Zingiberales
Family - Zingiberaceae
Genus - *Boesenbergia*

Species - *Boesenbergia rotunda*

Fig. 6: *Boesenbergia rotunda* plant.

Boesenbergia rotunda, commonly known as Chinese keys, fingerroot, lesser galangal or Chinese ginger. It belongs to the family Zingiberaceae. The morphology of the plant has been well characterized with the presence of the small globular shaped central subterraneous rhizome. Several slender and long tubers will be sprouting in the same direction from the rhizome like the fingers, from which the local name fingerroot came. Mainly, cultivated in India, Sri Lanka, China and Southeast Asia. It is used as common edible ingredient in many Asian countries such as Malaysia, Thailand, Indonesia, India, and China.

This species has 8 different botanical names which are *Boesenbergia cochinchinensis (Gagnep.) Loes., Boesenbergia pandurata (Roxb.) Schltr., Curcuma rotunda L., Gastrochilus panduratus (Roxb.) Ridl., Gastrochilus rotundus (L.) Alston, Kaempferia cochinchinensis Gagnep., Kaempferia ovate Roscoe,* and *Kaempferia pandurata Roxb*. Currently, it is known as *Boesenbergia rotunda (L.) Mansf*. (Tambunan and Alamudi, 2010).

Uses

Generally, cultivated at small home ranches and used as a condiment in food such as curry and soup because of its aromatic flavor, which is used to encourages appetite. Nearly, a hundred of bioactive compounds were successfully isolated and elucidated, consisted of flavonoid derivatives, chalcone derivatives, essential oils, ester, kawains, terpenes and terpenoids.

Fingerroot contains 1-3% of an essential oil. Several aroma components have been identified, from which 1-8 cineol, camphor, d-borneol and methyl cinnamate being the most significant. Trace components includes d-pinene, zingiberene, zingiberone, curcumin, zedoarin and others. From various studies, the rose-flavoured monoterpenoid alcohols geraniol and nerol are studied.

Many other bioactive compounds in *B. rotunda* includes Panduratin A, Pinostrobin, Pinocembrin Cardamonin, Boesenbergin A, Boesenbergin B, Alpinetin, essential oils, (2S)-6-Geranylpinostrobin, Geranyl-2,4-dihydroxy-6phenethylbenzoate,2′,4′-Dihydroxy-3′-(1′-geranyl)-6′-methoxychalcone,1′R,2′S,6′R)2-Hydroxyisopanduratin A, Tectochrysin,(±)-6-Methoxypanduratin A, (2R)-8Geranylpinostrobin, (2S)-7,8-Dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″ pentenyl)-8-phenyl-2H, 6H-benzo[1,2-b:5,4-b′]dipyran-6-one, 5,6-Dehydrokawain, Flavokawain C, Nicolaioidesin B, Isopanduratin A1, Isopanduratin A2 and Polyphenols (Yothin, 2016; Koller, 2009).

Fig. 7: Rhizomes of *Boesenbergia rotunda.*

The compounds were used as a traditional medicine to treat rheumatism, muscle pain, febrifuge, fever, gout, gastrointestinal disorders, flatulence, carminative, stomach ache, dyspepsia, and peptic ulcer. The fresh rhizomes are used to treat inflammatory diseases, such as dental caries, dermatitis, dry cough and cold, tooth and gum diseases, swelling, wounds, diarrhoea, dysentery, and as a diuretic (Koller, 2009).

B. rotunda is mainly propagated by the vegetative method using rhizome that are protracted for large scale multiplication. The conventional method might cause transmission of soil borne pathogens especially endophytic bacterial and fungal that can spread to other plants

and farming areas. Thus, the application of plant biotechnology approach in plant propagation is a simple and cost-effective way to obtain rich uniform planting materials. With that the standardization of optimum environmental parameters like light intensity, soil media, and other abiotic stresses are also essential for highest yield production with high quality of plants in the field. Further, the soil quality control such as aggregate stability, mineral contains, pH and salinity were also important in determining the growth development of plants have reported the influence of different substitute media on morphology and physiology changes on the plant (Yothin, 2016; Yusuf *et al.*, 2011).

With the addition to lotions for rheumatism and muscular pains, rhizomes are used as tonics to women in mixtures after childbirth, and as pastes for application to the body after confinement. About biological activity, *B. rotunda* exhibits antimutagenic, antibacterial, antifungal, analgesic, antiparasitic, antipyretic, antispasmodic, anti-inflammatory, anticancer, antioxidant, antiulcer, anti-dengue viral, anti-herpes viral activities, wound healing and insecticidal properties (Chawla *et al.*, 2014; Isa *et al.*, 2012; Ongwisespaiboon and Jiraungkoorskul, 2017).

Boesenbergia rotunda (L.) Mansf. and *Myristica fragrans Houtt.*, have been used to treat dyspepsia and peptic ulcer in Thai Traditional Medicine. *In vitro* anti-*H. pylori* testing had been performed with pinostrobin and red oil from roots of *B. rotunda*, and dihydroguaiaretic acid from arils of *M. fragrans* shown good potential for further drug development for treatment of dyspepsia and peptic ulcer (Koller, 2009; Yuliana *et al.*, 2013).

The fragment-based design of two natural product extracts and one synthetic inhibitor with panduratin A with competitive inhibitory activities to DENV-2 NS2B/NS3 had been studied. This approach is involved in dividing the ligand into separate fragments and docking the fragments into the active site of serine protease. Thus, a new ligand has been designed by linking the fragments and re-docking them into the active site of DENV-2 NS2B/NS3 serine protease with His51, Asp75, Ser135, and Gly153 in the binding pocket shown a good strength to become viable dengue inhibitors (Frimayanti *et al.*, 2011; Swain and Dudey, 2013).

The enzymatic assays were carried out using the fluorogenic peptide Boc-Gly-Arg-Arg-MCA, which is an active substrate of the DENV-2 NS3 protease used to study the activities of phytochemicals against NS3 with different concentrations. In these, all compounds

inhibited NS3 protease activity in a concentration dependent manner. From which, cyclohexenyl chalcone derivatives such as Pinostrobin and Cardamonin appeared to function through a non-competitive mechanism, while compounds Panduratin A and 4-hydroxypanduratin A presented as competitive inhibition (Kadir *et al.*, 2013; Oliveira *et al.*, 2017; SAMBASIVAN, 2009; Md-Mustafa *et al.*, 2014; Prashantha *et al.*, 2013).

Pinostrobin plays a role in elevating the activity of an antioxidant enzyme by reducing estrogen induced cell proliferation, mediating reduction of inflammation, decreasing spontaneous contractions of intestinal smooth muscle, elevating the activity of quinone reductase. Additionally, used as antispasmodic agent to inhibit aromatase activity. It also exhibited cytoprotective effects which induce antiulcerogenic property on rat (Bharti, 2014; Pigili and Runja, 2014).

The isolated cardamonin displayed antiviral activities, used to inhibit HIV-1 protease activity. Also, flavonoid panduratin A was found to reduce the development of human breast cancer and human colon adenocarcinoma cell, anti-aging activity by treating skin aging affected by UV, treating obesity and associated metabolic disorder, anticariogenic agent, and antioxidant activities for inhibition of lipid peroxidation in brain (Rashid, 2015).

Three flavanones, pinostrobin, pinocembrin, and alpinetin, and four chalcones, pinostrobin chalcone, pinocembrin chalcone, and cardamonin, had been used to automated docking towards dengue virus 2 NS2B/NS3 protease using 2FOM structure to understand the interactions of these reported inhibitors with protease. Through study, it was reported that the estimated free energy of binding for the flavanones were lower than those of their chalcone derivatives. Through SAR analysis, it was also suggested that the higher non-competitive inhibitory activity shown by pinostrobin was compared with other compounds possibly accounted by H-bonding interaction with the backbone carbonyl of Lys74, which is bonded to Asp75, which is one of the catalytic triad residues. The C5 hydroxyl and C7 methoxy groups on ring A, and the phenyl ring B was accounted to be important features for designing new compounds with potential inhibitory activities against DENV-2 NS2B/NS3 protease. Another docking study was studied using 2FOM structure with homology model of DENV-2 NS2B/NS3 protease with inhibitors, 4-hydroxypanduratin A and panduratin A as reference compounds. These derivatives were then used as ligands for docking, and afterward new competitive inhibitors were designed based on the docking result. This approach was

proposed to be an early stage drug discovery for identifying drug candidate (Tambunan and Alamudi, 2010; Qadir *et al.*, 2015).

Cucurbita pepo

Kingdom - Plantae
Subkingdom - Tracheobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Dilleniidae
Order - Violales
Family - Cucurbitaceae
Genus - *Cucurbita*
Species - *Cucurbita pepo*

Fig. 8: *Cucurbita pepo* plant.

Cucurbita pepo is also known as pumpkin and winter. It has a wide variety of uses, mainly as a food source and medicines. It is an herbaceous plant of the genus Cucurbita and the family of Cucurbitaceae. It is found in Russia, China, Italy, Mexico, India, Egypt, Iran, and USA (Yukes and Balick, 2010). Pumpkin are also named as Kumbra, Kohlu, Kaddu, Kumbala, Paarimal, Mathan or Chakkara kumbalanga, Lal bhopla, Kakharu, Sitaphal, Purangikkai or Pooshanikai, Gummadi kayi, Dangaree (Rajasree *et al.*, 2016).

It is a climbing annual plant that can grow to varying heights of 2m long or longer and has sprawling, prickly stems. Leaves are typically 5-lobed with spiky margins. Flowers are bright orange-yellow in color. Fruits are large and roundish with a tough rind in orange, yellow or

white flesh and yellow or cream-colored skin (Yukes and Balick, 2010; Teppner, 2004). Agricultural pumpkins are monoecious and mainly they are long crawl vine on the ground. The family *Cucurbitaceae* covers about 80 genera and over 800 species. It includes *C. pepo, C. mixta, C. maxima Duchesne, C. Ficifolia,* and *C. moschata.* From these, *C. pepo, C. maxima Duchesne* and *C. moschata Duchesne* have great economic and social value with high production world widely (Jafarian *et al.*, 2012). *C. pepo* is related to the winter type of cucurbita, but *C. maxima Duchesne* are more delicate and eaten freshly soon after harvest.

Uses

It is used for Cholesterol Lowering, Kidney function, fever, Laxative, Enuresis, Burns etc. The seeds are traditionally prepared as an infusion and occupied orally for diarrhea, intestinal parasites and worms. Also, fruit pulp prepared as a drink for the common cold and flu. Safety. Many research studies concluded it as anti-arthritic, antidiabetic, antihypertension, antitumor, antibacterial, anti-prostatic, antifungal, antihypercholesterolemia, intestinal antiparasitia, immunomodulatory, anti-inflammatory, antiviral, analgesic urinary disorders, anti-ulcer, and antioxidant (Jafarian *et al.*, 2012; Gutierrez, 2016; Bardaa *et al.*, 2016).

Plants have a long history in curing diseases, cancers and medicinal purposes. The first search for anticancer agents had been done in 1950’s when vinca alkaloids "Vinblastine and Vincristine" were discovered and cytotoxic podophyllotoxins were isolated (Colagar and Souraki, 2011).

Pumpkin was first cultivated in Mexico in 5500 years B.C. when beans and corn were used as the main food of North Americans. Further, in 1492 Christopher Columbus reported the implant of pumpkin for the first time in his journey and afterwards Gasper de Espinosa reported the implant of pumpkin during his journey, and at that time he named it Indian water-melon as very delicious and can be used in curing many diseases. From that time, pumpkin cultivation started in Europe (Yukes and Balick, 2010; Teppner, 2004; Gutierrez, 2016).

The enhancement of splenic lymphocyte proliferation, natural killer cell activity and an increase in the number of CD4+, CD8+ T cells and the CD4+/CD8+ ratio using pumpkin extracts has been studied. A model of cell-mediated response using delay type hypesensitivity (DTH) was studied through *in vivo*. In these, DTH reaction were computed by measuring the

amount of the paw swelling after inoculation of antigen to analyse useful pumpkin extract (Jafarian *et al.*, 2012).

Plant includes root, flower, leaf, fruit, and seeds. Plants skin and flower are useful for healing wounds like burning wounds. Further, flower infusion is used for sore throat or angina. Also, they can be used in salads with other vegetables (Colagar and Souraki, 2011).

Plants seed are consumed either roasted or raw. Also, used in cooking and baking as an ingredient in cereals, bread, cakes and salads. Also includes various components such as p-aminobenzoic acid, γ-aminobutic acid, polysaccharides, peptides, proteins, carotenoids as lutein, lutein epoxide, 15-cis-lutein (central-cis)-lutein, 9(9')-cis-lutein, 13(13')-cislutein, α-carotene, β-carotene violaxanthin, auroxanthin epimers, flavoxanthin, luteoxanthin, chrysanthemaxanthin, α-cryptoxanthin, β-cryptoxanthin (Gutierrez, 2016). The pumpkin seeds produce about 50% oil, the main constituents are Δ7 sterols such as avenasterol, spinasterol and sitosterol, stigmasterol as Δ5sterol. It also contains Triterpenoids, Sesquiterpenoids, Squalene, Tocopherols, Carotenoids, Minerals mainly (phosphorus, potassium, magnesium, calcium, iron, zinc and trace elements), Proteins and amino acids, Carbohydrates, Vitamins such as (thiamine, riboflavin, niacin, pyridoxine, and pantothenic acid), Phenolic glycosides, and Lignans (Rajasree *et al.*, 2016).

Unsaturated fatty acids, omega-6 and omega-9 convert into prostaglandin and then to thromboxan, these hormones help to increase HDL and prevent deposit of fat in arteries, which decrease the risk of a heart stroke. Pumpkin seeds possess oleic acid and linoleic acid which shows beneficial effects in hypertrophy condition. Vitamin E and vitamin A are antioxidant and inhibit free radical action, which helps in preventing cancer, especially prostate cancer. Minerals are useful for intestinal and bladder infections. Pumpkin seed contains phytosterols and cytostrols, which shows anticancer properties. Also, seed has been recognised to help in preventing prostate and colon cancer. Further, shown action against intestinal parasites, both tapeworms and roundworms, which are very common helminthes which can be eliminated by intake of seeds. Pumpkin contains special amino acid known as Cucurbitin in seeds also known as (-)-3-amino-3-carboxypyrrolidine. It can be used as anti-helminthic, and can eliminate worms (Colagar and Souraki, 2011).

Many study reported that carotenoid from pumpkin is known for keeping the immune system of an individual strong and healthy. Betacarotene is a powerful antioxidant as well as an anti-

inflammatory agent from pumpkin. It prevents accumulation of cholesterol on the arterial walls, which reduce chances of strokes. Presence of high alpha-carotene composition, pumpkin is supposed to slow down the process of aging and prevent cataract formation. Pumpkins is also known to reduce the risk of macular degeneration, a serious eye problem which can results in blindness. The high amount of fibre is good for the bowel. Pumpkin is associated with lowering the risk of hypertension with the help of potassium. Zinc in pumpkins helps to boosts up the immune system and improves the bone density. Pulp of pumpkin applied to scalds, inflammations, abscesses, boils and additionally, used for migraine, neuralgia, haemoptysis and hemorrhages (Teppner, 2004; Gutierrez, 2016).

The seed or seed extracts have been investigated in human clinical trials, helps to improve urinary symptoms of benign prostatic hyperplasia and inhibit urolithiasis. In animal studies, the plant or seed extract has shown antiallergenic and hepatoprotective effects. Through i*n vitro* study, compounds isolated from the seed have shown antiproliferative activity. Fruit and flower are an important source of pro-vitamin A and the seeds are good source of L-tryptophan. For common cold and flu, the fruit pulp is combined with the pulp of passion fruit and boiled to make a tea or beverage.

A combined extract of areca nut and pumpkin seeds gave an excellent result in heterophyiasis. Study of pumpkin leaf extract with different solvents showed good result against larvicidal, ovicidal and repellent activities against *Culex quinquefasciatus* (Etewa and Abaza, 2011). Some of the recent reports showed the expression of dengue virus domain III in maize seeds and *Cucurbita pepo*. But, the immunogenicity of these plants still needs to be study in animal models.

In research studies, isolated cucurbitin, one of the pharmacological constituents of plant has confirmed a low toxicity level in both humans and dogs. Pumpkin seeds are very beneficial because of their delicate oils, and can be stored in such a way that they are protected from light and moisture to reduce oxidation (Yukes and Balick, 2010; Waheed *et al.* 2016).

CONCLUSION AND FUTURE DIRECTIONS

Current communication gives an insight of pathology, epidemiology, complications and diagnosis with special reference to potential plants and having good potential to be discovered as new drug candidates for dengue fever as found in *Carica papaya*, *Euphorbia hirta* and other medicinal plants.

This review has covered three potential plants and their active phytochemicals that could be used in the treatment of dengue. This study highlights the information gathered for various parts and extract from plants for the treatment of dengue. However, there were many other plants that have not yet been fully explored, which may contain a broad range of potential therapeutic applications. But, the problems mainly arise when the four related dengue serotypes are present which show noticeable differences in their inhibition profiles. Thus, various strategies have been modified to develop an effective vaccine or drug against dengue virus. To identify potential anti-dengue plants or compounds, knowledge of the mechanisms of virus infection need to be understand for the development of appropriate drugs. Further research is also needed to determine how to target the most appropriate phase to prevent the spread of virus infection. Based on these, one can target different phases such as: (1) infection of host cells, (2) the viral maturation process, (3) synthesis of viral RNA, and (4) the spread of viral particles.

Many epidemiological and animal models have been investigated for cellular and sub-cellular targets for these antivirals and promising results have been observed which can be further analysed using *in vivo* study. Number of synthetic antiviral drugs are available, which proves to be effective against viruses but then again in a specific manner. Yet again, the problem of anti-viral resistance is still there. Thus, knowledge based on traditional system of medicines can be used in the development of various phytochemicals from different medicinal plants.

Thus, a potential role of the extracts of *Psidium guajava*, *B. rotunda* and *Cucurbita pepo* in improving the platelet counts in different thrombocytopenic disorders including a role in upgrading the haemorrhagic complications of dengue fever. The isolated bioactive compounds from *B. rotunda* have high potential in treating many diseases. In the future with the development of different techniques, a successfully plant-based drug will be developed. Molecular, cellular and other field progressions encourage to accelerate the development of better and nontoxic drugs to treat the diseases. Many compounds from *Cucurbita pepo* mainly helps to maintain good immune system with low toxicity level. It is also used to prevent cancer.

There should be an extensive association among academic research groups, clinicians, and industries throughout the globe so that ethnobotanical knowledge can be used and finally converted into effective drug against dengue virus.

The development of new anti-dengue candidates from bioactive compounds is needed to find out effective and less toxic drugs. Therefore, any extensive study of the plants with isolated active compounds that have shown anti-dengue activity should go further for additional *in vitro* and *in vivo* animal testing, toxicity analysis and clinical tests. This can be helpful to reveal a promising compound to be optimized and used in the production of new anti-dengue compounds. Phytochemicals from medicinal plants may prove as valuable to the health of individuals and for the nation. Such discoveries lead to the development of highly efficient and nontoxic anti-dengue compounds.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

COMPLIANCE WITH ETHICAL STANDARDS

On behalf of all the authors, the corresponding author ensured compliance of ethical rules applicable for publishing this manuscript in the journal.

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