ORIGINAL ARTICLE

Dengue and drawbacks of marketed *Carica papaya* leaves supplements

Nusrat Hossain[1], Palanirajan Vijayaraj Kumar[1], Yeong Siew Wei[2]

[1]Faculty of Pharmacy, Department of Pharmaceutical Technology, UCSI University, Kuala Lumpur Campus (South Wing), No. 1, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras 56000, Kuala Lumpur, Malaysia, [2]Faculty of Pharmacy, Department of Clinical Pharmacy, UCSI University, Kuala Lumpur Campus (South Wing), No. 1, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras 56000, Kuala Lumpur, Malaysia

Abstract

Aim: This review is mainly focused on describing deadly disease, dengue and the main drawbacks related to marketing *Carica papaya* leaves supplements. **Materials and Methods:** Information was collected from various published research, review articles, web pages, and other online databases related to the topic. Dengue is a deadly viral disease and it has affected 40% of the world population. It can be transmitted by mosquitoes (*Aedes aegypti*) infected with one of the four dengue virus serotypes. Due to this complex virus serotype, there is no particular therapeutic treatment for dengue. Therefore, despite of the severity and deadly effects of dengue, vaccines are not available. **Results and Discussion:** As a result, the use of herbal supplementary medicines has been increased in passing time. The supplements of *C. papaya* leaves have shown promising prospect due to the platelet increasing property. However, the manufacturing techniques used in several marketed *C. papaya* leaves formulations can degrade the active phytochemicals, which will suppress the beneficial effects of the supplements. **Conclusion:** There is a need to improve the old herbal formulations for the current crisis. Therefore, the drawbacks of marketing *C. papaya* leaves supplements need to be modernized for better patient care.

Key words: *Carica papaya* leaves, dengue, supplements

INTRODUCTION

Dengue is a severe arthropod-borne viral disease which occurs mainly in tropical areas and it can cause both morbidity and mortality in humans. *Flavivirus* is the main vector for spreading dengue among human.[1] According to the survey of the World Health Organization (WHO), dengue has affected 40% of the total global population. However, dengue epidemic has extended many new countries and amplified significantly in the already affected areas.[2] Being a tropical country and having humid weather condition, the people of Malaysia are suffering from an increased number of dengue cases.[3]

According to the Health Ministry of Malaysia Guideline for the management of dengue, the frequency of Case Fatality Rate of dengue was higher in the year 2007 compared to 1999 in Malaysia.[3] In 2014, the WHO declared that 80,578 dengue cases had been reported in Malaysia and among them 153 patients died.[4] Dengue treatment can be both symptomatic and supportive. Due to the absence of any particular therapeutic treatment, dengue can be controlled with only proper supportive care and vigilant fluid administration during different phases.[5] The main complications of dengue hemorrhagic fever are plasma leakage, hemoconcentration, and abnormalities in homeostasis. Plasma leakage occurs due to the activation of infected monocytes and T-cells. This will cause endothelial cell dysfunction and cause plasma leakage in dengue patients. Plasma leakage in dengue patients will hamper the blood clotting and it can cause death. By increasing the platelet count in the patient, the death rate can be reduced.[6]

The WHO fact data sheet, published in December 2014, stated that the people of some Asian and African countries

Address for correspondence:
Palanirajan Vijayaraj Kumar, Faculty of Pharmacy, UCSI University, Kuala Lumpur Campus (South Wing), No. 1, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras 56000, Kuala Lumpur, Malaysia.
E-mail: palanirajan@ucsiuniversity.edu.my

Received: 14-12-2015
Revised: 09-02-2016
Accepted: 16-02-2016

depend on cultural or traditional treatments for dengue as supportive care.[7] Recently, *Carica papaya* leaves juice has become popular among the dengue suffering patients due to the platelet increasing activity of the leaves.[8,9] Various active phytochemicals (papain, flavonoids, phenols, saponins, alkaloids, ascorbic acid, etc.) had been identified in *C. papaya* leaves by the phytochemical screening process.[10-12] Based on these findings and clinical trials, physicians are advising dengue patients to take fresh *C. papaya* leaves juice while dengue treatment.[9] However, during the rainy season, dengue spreads rapidly compared to other time of the year. Tropical countries, such as Malaysia, India, Thailand, Indonesia, Sri Lanka, and the Philippines, suffer more due to the humid environment, which is preferable for the fertilization of *Aedes* mosquitoes.[13,14] Therefore, the sudden increase of patients in these areas makes the availability of fresh *C. papaya* leaves slightly difficult. Furthermore, fresh juice extraction on a daily basis and bitter taste of the juice are the main drawbacks of the treatment.

MATERIALS AND METHODS

Search Criteria

Information was collected from various published articles, review articles, and web pages related to the topic. Original published resources of recent research on dengue, the medicinal activity of *C. papaya* leaves and drawbacks of available supplements of *C. papaya* leaves were transferred from PubMed, Science Direct, Research gate, WHO guidelines, and other online databases. The search criteria were restricted to dengue, brief description of *C. papaya* leaves and current marketed supplements of *C. papaya* leaves.

Dengue Virus (DV)

Dengue is an endemic disease.[15] It has affected the normal life of people living tropical and subtropical regions. Dengue is the most common human arthropod-borne virus disease and it causes thousands of deaths every year.[16] DV is very unique in structure. It can be transmitted by mosquitoes named *Aedes aegypti*. *Aedes* mosquitoes are normally small and black with the white lines on the body and legs. The DV belongs to the Flaviviridae family and it has four different serotypes (DV-1 to DV-4).[17]

The DV is composed of mainly a nucleocapsid. It is covered by glycoproteins and totally surrounded by a lipid membrane.[15] It consists of a positive-sense genome and single-stranded ribonucleic acid (RNA). The RNA has encoded with a polyprotein precursor of the main viral proteins. This precursor can be cleaved by host and viral proteases. They are consist of three structural and seven non-structural proteins. The structural protein is capsid C, pre-membrane, and envelope E.[18] The non-structural proteins are NS1, NS2a, NS2b, NS3, NS4A, NS4B, and NS5.[19] Schematic representation of DV is shown in Figure 1.

The three structural proteins are united into the developed infective virion. However, the non-structural proteins only help in the replication of the virus. Researchers have isolated DV from the macrophages, polymorph nuclear leukocytes, dendritic cells, and others. Furthermore, it has also been identified in megakaryocyte progenitors and platelets. These findings from different journals propose that DV has the capability to react directly with megakaryocytes and platelets, which may induce thrombocytopenia.[15,20,21]

Management of Dengue

The *A. aegypti* mosquito is the primary vector of dengue. *A. aegypti* can control *Flavivirus* transmission. *Flavivirus* has four separate serotypes, which are DV-1, DV-2, DV-3, and DV-4. However, the DV can transmit in human beings through the bites of infected female mosquitoes. After 4-10 days of virus incubation period, an infected mosquito is capable of transmitting the virus its whole life. The illness started to build up after the incubation period. It followed three main phases-febrile, critical, and recovery. In the febrile phase, suddenly high temperature can be identified as symptoms. In critical phase, the temperature of the patient falls to 37.5-38°C or less. The afterward platelet count is reduced, and it causes plasma leakage. In the recovery stage of dengue, the patient started to recover, and proper monitoring of symptoms should be done to next following 48-72 h.[6,13] Schematic outline of different phases of dengue are shown in Figure 2.

Febrile phase in dengue can last for 2-7 days. The main symptoms are a headache, skin erythema, arthralgia, body ache, myalgia, and facial flushing. In the beginning of the critical phase, hematocrit levels may increase. However, in the critical phase platelet count started to lower down and it causes plasma leakage. In dengue patients, shock occurs due to plasma leakage. During 24-48 h of critical phase, the fluid management of the patient is very critical. After 72 h of critical phase, recovery phase started. However, in recovery phase; patient's physical condition started to improve. In this phase, the normal appetite will return, gastrointestinal bleeding will reduce, the platelet count will increase, and blood pressure will stabilize. Bradycardia and electrocardiographic changes can be identified during the recovery stage.[22] Schematic depiction of the symptoms of dengue fever is shown in Figure 3.

According to the guideline of the WHO, dengue management is done using three steps, which are overall assessable of the disease, diagnosing disease phase with severity, and finally, focus on the treatment of dengue. Clinical diagnosis can be done by serological testing in the febrile phase of dengue.[13]

In the febrile phase, patients are only encouraged to take paracetamol and oral fluid saline. However, in the critical

phase 0.9% saline is given to dengue patients. If the symptoms started to worsen and hematocrit level is rising, the fluid infusion rate needs to be increased. In critical phase, patients started to show more symptoms than the febrile phase. They may experience with bleeding from the gums, epistaxis, face, and vagina. At the beginning, patients' do not require blood transfusion. However, dengue patients' with significant bleeding need blood transfusion. It has been suggested that replacement red blood cells of 5-10 ml/kg or replacement blood of 10-20 ml/kg can be given with proper care. Non-shock dengue patients are encouraged to take oral fluid intakes and bed rest. However, transfusion of blood or fresh frozen plasma can be done in case of severe gastrointestinal bleeding. In the recovery phase, the rate of IV infusion is gradually reduced from 5 to 7 ml/kg/h for 1-2 h, and then to 3-5 ml/kg/h for 2-4 h. The IV fluid rate can be continued up to 24-48 h during the recovery phase.[6]

Vaccines are not available for immunization in dengue.[24] Therefore, the use of herbal-based medicine and medicinal plants such as *C. papaya* for alternative treatment in dengue is growing.[17] Papaya leaves contain active components such as papain, chymopapain, ascorbic acid, flavonoids, saponin, phenolic acid, alkaloids, and quercetin, and some of them can be degraded due to heat and storage condition.[25-28] Therefore, the heat inducing extraction methods such as micronization, fermentation, Soxhlet extraction can degrade the heat sensitive active phytochemicals of papaya leaves.[29,30] However, a formulation of lyophilized *C. papaya* leaves can protect this phytochemical degradation.

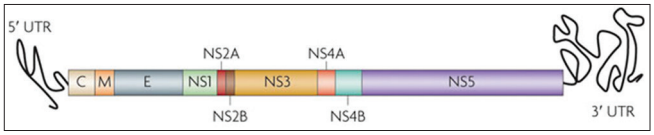

Figure 1: Schematic representation of dengue virus. Adapted from reference [13]

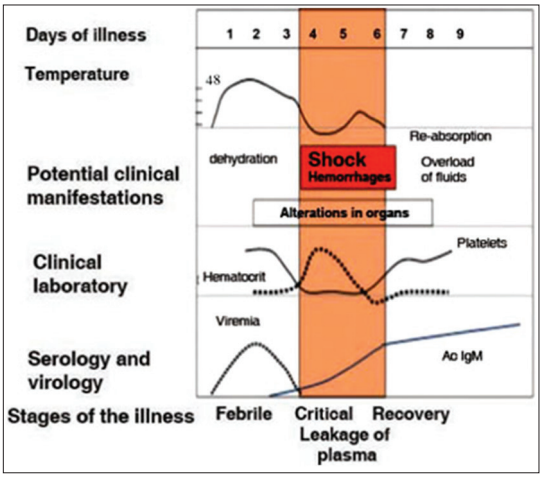

Figure 2: Schematic outlines of different phases of dengue. Adapted from reference [6]

Interaction of DV with Human Antibodies

In our body, the first immunological reaction against DV started by producing antibodies. Our immune system will produce antibodies against the envelope protein of DV. However, our body can react with DV by two different responses, which are primary and secondary responding.[13] An individual will show a primary response if they are affected by DV for the first time. But if an individual had suffered from previous DV infection, the secondary response can be identified. During the primary dengue infection, DV specific IgM and IgG antibodies will appear. In the secondary dengue infection, our immune system will produce a high amount of IgG antibodies as B-cells and memory B-cells will identify the virus during the onset period. However, DV will induce DNA-based immunization and the production of IgG1 and IgG3 subtypes of antibodies.[31,32]

An individual will develop serum antibodies against both structural and non-structural protein of DV. In primary infection, researchers have identified high levels of prM and NS1 antibodies compared to secondary infection. NS1 antibodies can react with the antigens of platelets and endothelial cells. It has been also reported that the production NS1 antibodies can cause vasculature in dengue.[31] The primary infection pathway of DV is shown in Figure 4.

Diagnosis of Dengue

Different diagnosis method is used to identify dengue infection, and they have their own advantages and disadvantages. Using virus detection method, DV can be isolated from the blood plasma. However, different virus serotypes can be identified using serotype-specific monoclonal antibodies. Viral RNA detection of dengue can be done by nucleic acid amplification test using the blood tissue of the dengue patient. Furthermore, reverse transcription-polymerase chain reaction (RT-PCR) or nested RT-PCR can be used to detect the DV.[34]

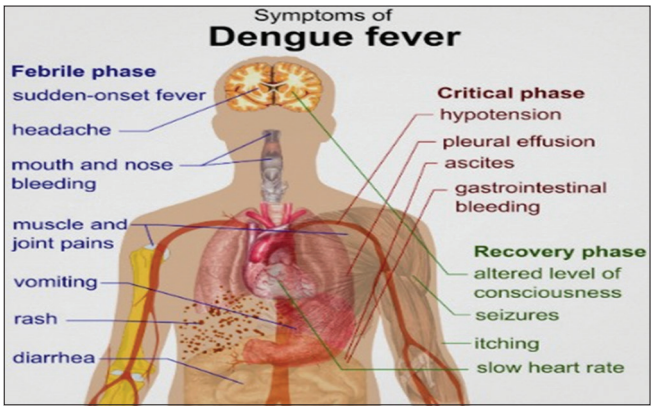

Figure 3: Schematic depictions of the symptoms of dengue fever. Adapted from reference [23]

Antigen detection can be done by either NS1-based assays or immunohistochemistry. For identifying NS1-based antigen in dengue infection, enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic assay are used. NS1-based antigen can be identified during the first 9 days of both primary and secondary dengue infection.[35] Schematic representation of the immunological response to dengue infection is shown in Figure 5.

Using the serological method, the production of IgM, IgG, and IgA can be identified. IgM antibody production is higher in primary dengue infection and, in contrast, IgG antibody level is higher in secondary dengue infection. During primary dengue infection, almost 93% of the patient produces a detectable IgM level of antibodies. Therefore, ELISA-based IgM assay is used to identify the primary dengue infection. IgG-based assay method can be used to identify both past and current dengue infection. Using this method; the level of infection (either primary or secondary) can be recognized.[34]

Botanical Descriptions of *C. papaya*

Papaya plant is normally found in tropical areas around the world. The family name is Caricaceae, and the botanical name is *C. papaya*. Papaya is a tree like a plant and it can be 16-33 feet tall. The leaves are 50-70 cm in diameter with deep seven lobes. *C. papaya* tree comes into fruiting within 5 months and lives for 4-5 years. Usually, male and female flowers are on different trees, but some flowers are bisexual. *C. papaya* leaves are spirally arranged. They are clustered near apex of the trunk; the petiole is up to 1 m long, hollow, greenish, and 25-75 cm in diameter.[12,37] In Figure 6, grown up *C. papaya* tree is shown.

Plants used in Dengue Treatment

In a review article (2013) of potential anti-dengue medicinal plants, Siti *et al.* mentioned that *Boesenbergia rotunda* (Chinese ginger), *Andrographis paniculata* (Hempedu Bumi), *Azidarachta indica* (neem), *C. papaya* (papaya), *Cladosiphon okamuranus* (seaweed), *Gymnogongrus griffithsiae* (red seaweed), *Momordica charantia* (bitter melon), *Quercus lusitanica* (oak), *Euphorbia hirta* (tawa-tawa), *Rhizophora apiculata* (Bakau), *Ocimum sanctum* (Tulsi), etc., were reported to be used for traditional treatment of dengue.[17] Among these plants, papaya leaves have been used in several studies for the treatment of dengue.[23] Several animal and human clinical trials had been conducted with *C. papaya* leaves juice for elaborating the toxicity and platelet inducing activity.[9,38]

Phytochemical Constituents of *C. papaya* Leaves

The medicinal effects of *C. papaya* leaves extracts can be recognized due to the presence of several active components such as papain, chymopapain, alkaloids, glycosides, tannins, saponin, L-tocopherol, ascorbic acid, riboflavin, flavonoids, and minerals (such as calcium, iron, magnesium, potassium, sodium, and zinc).[37,39] The phytochemical constituents of *C. papaya* leaves in different solvents and mineral composition are shown in Tables 1 and 2.

Afzan *et al.,* 2012 has done the phytochemical analysis of *C. papaya* by UPLC-Triple TOF-ESI-MS (ultra-performance liquid chromatography time-of-flight mass analyzer with an electrospray ionization source) fingerprinting.[43] Canini *et al.*, 2007 has identified the phenolic compounds of *C. papaya* using gas chromatography.[44] High-performance

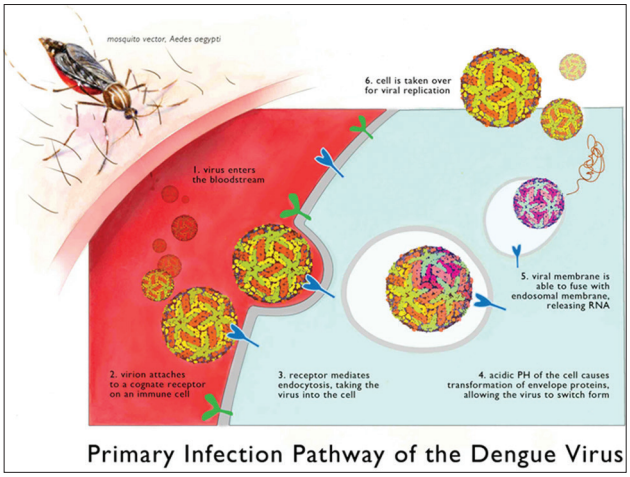

Figure 4: Schematic representation of infection pathway of the dengue virus. Adapted from reference [33]

Table 1: Phytochemical constituent of *Carica papaya* leaves in mg/10 g residue of different solvents

Phytochemicals	Solvent extraction (ethanol) mg/10 g residue	Solvent extraction (hot water) mg/10 g residue	Solvent extraction (acetone) mg/10 g residue	Solvent extraction (chloroform) mg/10 g residue
Phenols	0.7	0.7	0.64	0.56
Flavonoids	10.0	0.0	0.21	0.22
Saponins	0.8	0.04	0.04	0.4
Alkaloids	0.6	0.01	0.02	0.54
Glycosides	0.7	0.01	0.3	1.6

Adapted from references [40-42]

liquid chromatography (HPLC)-based activity profiling of *C. papaya* has been done by Julianti *et al*.[45] High-performance thin layer chromatography phytochemical screening has been done by Anjum *et al.*, 2013 using different extracts of *C. papaya* leaves. However, fingerprinting was done by determining the r_f values of the prepared extracts.[46] Flavonoids of *C. papaya* leaves have been investigated using aqueous and ethanolic extract using HPLC method. In the HPLC, rutin and lycopene (between 190 and 700 nm) were used as a standard. In this research, 95% methanol and 5% water was used as a mobile phase. However, 1% phosphoric acid was also added to maintain the pH range within 4.0. All the solvents were degassed before use by sonication.[47]

In a recent study, Fadare *et al.*, 2015 attenuated total reflectance Fourier transform infrared spectroscopy (FTIR) and HPLC spectroscopic studies have been done using both *C. papaya* leaves and flowers. For HPLC analysis, they have used mobile phase containing 90% phosphate buffer, 8% acetonitrile, and 2% methanol. In FTIR spectrum, they identified the functional groups of *C. papaya* leaves and discovered the active components based on the absorption band values in the region of infrared radiation.[48]

Medicinal Activity of the Phytochemicals of *C. papaya* Leaves

Researchers found that phytochemicals of *C. papaya* leaves like Vitamin C had the capability of treating idiopathic thrombocytopenia.[49] Otsuki *et al.*, 2010 found the anti-tumor activity of *C. papaya* leaves in an animal model.[50] In a recent study, Panzarini *et al.,* 2014 explained the beneficial use of *C. papaya* leaves extracts as an antioxidant and anticancer agent.[51] Juárez-Rojop *et al.*, 2014 identified the hypoglycemic activity of papaya leaves in diabetic rats.[52] The anti-inflammatory effect of *C. papaya* leaves extracts in a murine model was found by Gamulle *et al.,* 2012[53] and the anti-plasmodial effect of papaya leaves are also identified by HPLC-based activity profiling.[45]

Table 2: Mineral composition of the *Carica papaya* leaves on dry weight basis (mg/kg)

Mineral	Green leaf (mg/kg)	Yellow leaf (mg/kg)	Brown leaf (mg/kg)
Calcium	8612.50	3762.50	4362.50
Magnesium	67.75	28.55	35.35
Sodium	1782.00	567.00	324.00
Potassium	2889.00	819.00	468.00
Iron	90.50	147.50	79.50
Manganese	9.50	5.00	4.50
Ascorbic acid	162.9	96.2	112.6
Riboflavin	1.3	0.4	0.6
Thiamine	9.4	4.1	5.2

Adapted from reference [39]

Owoyele *et al.*, 2008 has identified the anti-inflammatory action of *Carica papaya* leaves using a cold maceration extraction method using 2 L of ethanol.[54] *C. papaya* leaves were individually extracted using 95% of cold ethanol, cold methanol, and pure water to identify the antimicrobial activity by Anibijuwon and Udeze in 2009.[42] Cardiovascular Effects of *C. papaya* leaves have been studied using alcoholic extract by Gupta *et al.,* 1990. They have identified that leaves extract between the doses of 100 and 200 mg/kg can give total protection against electroshock-induced spasms in rats.[55] Lyophilized *C. papaya* leaves juice (2.6% w/w yield) have been used to determine the toxicity using Sprague-Dawley rats. In the study, Sprague-Dawley rats at doses around 0.01, 0.14, and 2 g/kg of *C. papaya* leaves extract were administered. After administering the dose, mainly general behavior, clinical signs, hematological parameters, serum biochemistry, and histopathology changes were analyzed. A dose until 2 g/kg was found to be safe among the rats.[43]

Using 400 mg/kg *C. papaya* leaves in a rat model, it was found that it can significantly increase the platelet count and decrease the blood clotting time in 7 days.[38] In a recent docking study, Senthilvel *et al.,* 2013 found that flavonoids (quercetin, protocatechuic acid, p-coumaric acid, caffeic acid, chlorogenic acid, kaempferol, and 5, 7-dimethoxycoumarin) from *C. papaya* inhibits NS2B-NS3 protease and can prevent dengue viral assembly. Complex formation between NS3 and

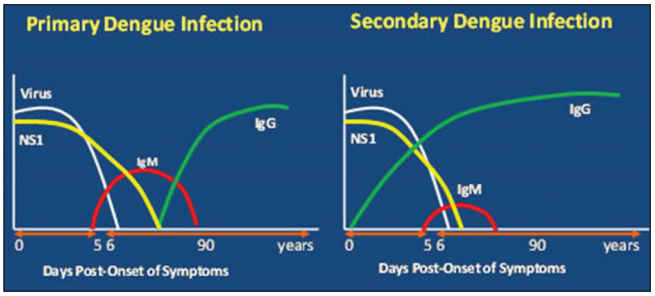

Figure 5: Schematic representation of immunological response to dengue infection. Adapted from reference [36]

Figure 6: *Carica papaya* tree. Adapted from reference [12]

NS2B co-factor is needed for virus replication. The active components of *C. papaya* leaves can control this process.[56] *C. papaya* leaves have membrane-stabilizing properties, and they can control the damage of blood cells.[41]

Papaya Leaf Extract in Dengue Treatment

Ahmad *et al.* have done a study using the case report of a 45-year-old male patient, suffering from dengue. It was observed that the platelet count increased from 55 × 10^3/μL to 158 × 10^3/μL in the patient by administering 25 ml of *C. papaya* leaves extract for 5 days.[57] Dharmarathna *et al.* found that papaya leaves can increase the platelet and RBC count in the test group compared to the control group of animal.[8] Toxicological studies of *C. papaya* leaves extract had been done using Sprague-Dawley rats by Afzan *et al.*, 2012. The results suggest that C. *papaya* leaves extract up to 2 g/kg was safe for traditional use in the animal model.[43]

A patent had been done by Stanbridge *et al.*, 2014 about the application of *C. papaya* leaves extract inhibiting hypoxia-inducible factor activity. In the research, it was proved that papaya leaves can treat hypoxia-related conditions or diseases such as inflammatory diseases, vascular diseases, cancer, and infections.[58]

Arachidonate 12-lipoxygenase (ALOX 12) Gene Expression in Platelet Production

In humans, normal platelet count ranges from 150,000 to 350,000. Thrombocytopenia occurs if platelet count reduces and it may cause plasma leakage in dengue patients. Megakaryocyte is responsible for the production of blood platelets, and ALOX 12 genes are strongly expressed in megakaryocytes. ALOX 12, which is also known as platelet-activating factor receptor (PTAFR), plays an important role in platelet aggregation. It means that PTAFR gene can be a precursor for platelet production.[59]

In a clinical trial, *C. papaya* leaf juice was given to 228 dengue patients, and the RNA was extracted for further investigation. It was found that ALOX 12 gene activity increased 15-fold in the experimental groups compared to the control days in 3 days.[9]

Marketed Formulation of *C. papaya* Leaves

Papaya leaf extract formulations are available on the global market as supplements under Rochway,[60] Iowa Select Herbal, Herbal Papaya[61,62], and SidoMuncul Herbal.[63] However, they are formulated using micronization, fermentation, and liquid extraction. Marketed *C. papaya* leaves supplements were shown in Table 3.

Problems with Micronized *C. papaya* Leaves Formulations

Rochway is one of the leading herbal supplements manufacturers of Australia and SidoMuncul is a fully-modernized herbal company in Indonesia. Both companies are currently producing micronized *C. papaya* leaves capsules. However, extraction methods such as milling and supercritical fluids used in micronization can cause mechanical stress, and it can modify the active compounds of any herbal product. The mechanical forces such as milling and grinding during micronization can also cause physical stress and degradation of the product. Specially, during micronization thermal stress occurs and this can degrade the heat sensitive compounds.[64] However, micronization with supercritical fluids such as CO_2 can be difficult, due to oxidative stresses.[65] The most noticeable drawback of supercritical fluids is the fact that several molecules (specially flavonoids) are not soluble in

Table 3: Marketed formulation of *Carica papaya* leaves with indication and dosage forms

Company name	Brand name	Dosage form	Indication
Rochway	Papaya leaf extract capsules	Each capsule contains 100% natural ingredients micronized papaya dry leaves	Supplement
Rochway	Papaya leaf extract – 500 ml	100% bio-fermented dry *Carica papaya* leaves extract	Supplement
Herbal Papaya	Papaya Leaf (Paw Paw Twig) Extract (alcohol-free)	60 Veggie capsules of dry papaya leave	Supplement
Iowa Select Herbs LLC	Papaya leaf extract 4 oz (120 ml)	Liquid extract of papaya leave	Supplement
SidoMuncul Herbal	Sari Daun Pepaya	Each capsule contains 500 mg of dry *Caricae folium* (papaya leaf) extract	Supplement
Celebration Herbals	Herbal tea, papaya leaf, caffeine free, 24 tea bags, 1.33 oz (38 g)	Tea bags (fermented papaya leaves)	Supplement
Micro lab limited	Caripill	Tablet containing dry *Carica papaya* leave extract	Supplement

Adapted from reference [60-63]

CO_2.[66] One way to overcome this problem is to change the supercritical fluid. However, this is not possible as the other supercritical fluids, for example, N_2O, light hydrocarbon, etc., is much more lethal and less environmental friendly than CO_2.[67]

Vandana *et al*., 2014 has mentioned that the main disadvantages of micronization technique could be controlling the uniform particle size of formulation and avoiding hydrophilic particles to form agglomeration.[68] Therefore, the marketed products of Rochway named micronized papaya leaf extract capsules and SidoMuncul Herbal capsules might face the problems regarding thermal degradation.

Problems with Fermented *C. papaya* Leaves Formulations

Rochway is one of the leading manufacturers of probiotics and herbal supplements in Australia. They have marketed bio-fermented *C. papaya* leaves extract named papaya leaf extract - 500 ml. However, the existing fermentation scale-up process cannot meet adequately the advance technical and economic demands of the modern industry. Therefore, alternative methods are developed for better commercialization.[69] However, in the fermented products hazardous microbial contamination always exists. David *et al.* found that the traditional fermentation process of fresh leaves of *Cassia obtusifolia* (known as sicklepod or Chinese senna) can decrease proteins, total fibers, and volatile fatty acid content.[70]

Therefore, marketed product of Rochway named papaya leaf extract - 500 ml might have the disadvantage of low active phytochemicals. However, purification of end products, balancing the yields, and biomass estimation are also major problems of the fermentation process, which influence the researchers to find other solutions for extraction.[71]

Problems with Liquid Extract of *C. papaya* Leaves

Herbal Papaya, Iowa Select Herbs, Hawaii Pharm, Rochway, etc., are producing *C. papaya* leaves liquid extract as supplements. The general liquid extraction techniques of the medicinal plant include hot continuous extraction (soxhlet), aqueous-alcoholic extraction, supercritical fluid extraction, and distillation techniques (water distillation, steam distillation, phytonic extraction with hydrofluorocarbon solvents).[72] However, different solvents are recommended for extracting specific active components. For example, ethanolic solutions are suggested for extracting tannins, polyphenols, polyacetylenes, flavonols, terpenoids, sterols, alkaloids, and chloroform solutions are suggested for terpenoids, flavonoids, phenols, etc.[73] In a previous comparison study of different extraction methods on spearmint leaves (*Mentha spicata* L.), Bimakr *et al.* found that total active flavonoid yield can be effected by the temperature.[74] In a review article, Shah *et al.* mentioned that for protecting the natural components of plant different modernized extraction methods need to be developed. Because the traditional drying techniques had various limitations such as lengthier extraction timeline, large solvent volumes, and degradation of thermolabile components.[75]

Therefore, it can be postulated that marketed liquid extracts of companies such as Herbal Papaya, Iowa Select Herbs, Hawaii Pharm, and Rochway might face all these limitations and the main drawback of the liquid extract will be the bitter taste of *C. papaya* leaves.

Problems with of *C. papaya* Leaves Tablets

Tablets are the most commonly used dosage form. Because, during large manufacturing simplicity and economic condition need to be considered. Therefore, recently Micro Lab Limited has introduced *C. papaya* leaves tablet (Caripill) in the market. Despite several advantages sometimes, certain herbal substances resist compression pressure and need a special formulation technique for improving bioavailability. For avoiding these problems, punching pressure or percentage of excipients is increased. However, high punching pressure may degrade the thermolable active components of herbal extract, and excess binding agent may slow the dissolution and disintegration of the formulation.[76,77]

In 2014, Pushpalatha *et al.* mentioned the detailed formulation process of Ashoka tablets with an industrial perspective. They have added microcrystalline cellulose, maize starch, colloidal silicon dioxide, and talc and magnesium stearate for preparing better granulating solutions. However, they dried the solution at 60°C and blended in high speed for several hours.[78] However, drying the herbal extract for long time can make them brittle and loose active components.[79]

However, similarly, the recently marketed *C. papaya* leaves tablet (Caripill) might face these problems during manufacture. Therefore, the company did not reveal any formulation process to the public. The use of excipients, punching pressure, and phytochemical protection was also not mentioned.

CONCLUSION

In recent years, dengue has become an epidemic in tropical countries. Unfortunately due to the absence of proper treatment method, the death rate is increasing. Researchers from several organizations and companies (example-Bill and Melinda Gates Foundation) are trying to discover a new vaccine for dengue. In 2014, Sanofi Pasteur released results of phase three trial having almost 10,275 children aged from 2 to 14 years in Indonesia, Malaysia, Philippines, Thailand, and Vietnam. But meanwhile, we need to improve the old

herbal formulations for the current crisis. Therefore, the drawbacks of marketed *C. papaya* leaves supplements need to be modernized for better patient care.

REFERENCES

1. Rajapakse S, Rodrigo C, Rajapakse A. Treatment of dengue fever. Infect Drug Resist 2012;5:103-12.
2. Balmaseda A, Hammond SN, Pérez MA, Cuadra R, Solano S, Rocha J, *et al.* Short report: Assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. Am J Trop Med Hyg 2005;73:1059-62.
3. Available from: http://www.moh.gov.my/attachments/5502.pdf. [Last accessed on 2014 Nov 07].
4. Available from: http://www.reliefweb.int/sites/reliefweb.int/files/resources/dengue_biweekly_21oct2014.pdf. [Last accessed on 2015 Feb 02].
5. Hung NT. Fluid management for dengue in children. Paediatr Int Child Health 2012;32:39-42.
6. Available from: http://www.whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf?ua=1. [Last accessed on 2014 Nov 07].
7. Available from: http://www.apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf. [Last accessed on 2015 Sep 09].
8. Dharmarathna SL, Wickramasinghe S, Waduge RN, Rajapakse RP, Kularatne SA. Does *Carica papaya* leaf-extract increase the platelet count? An experimental study in a murine model. Asian Pac J Trop Biomed 2013;3:720-4.
9. Subenthiran S, Choon TC, Cheong KC, Thayan R, Teck MB, Muniandy PK, *et al*. *Carica papaya* leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evid Based Compl Altern Med 2013;2013:1-7.
10. Akhila S, Vijayalakshmi N. Phytochemical studies on *Carica papaya* leaf juice. Int J Pharm Sci Res 2015;6:880.
11. Subramanian G, Tewari BB, Gomathinayagm R. Antimicrobial properties of *Carica papaya* (Papaya) different leaf extract against *E. coli*, *S. aureus* and *C. albicans*. Adv J Pharmacol Pharmacother 2014;1:25-39.
12. Macalood JS, Vicente HJ, Boniao RD, Gorospe JG, Roa EC. Chemical analysis of *Carica papaya* L. Crude latex. Am J Plant Sci 2013;4:1941.
13. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, *et al.* Dengue: A continuing global threat. Nat Rev Microbiol 2010;8 12 Suppl: S7-16.
14. Promprou S, Jaroensutasinee M, Jaroensutasinee K. Climatic factors affecting Dengue haemorrhagic fever incidence in Southern Thailand. Dengue Bull 2005;29:41-8.
15. Hottz E, Tolley ND, Zimmerman GA, Weyrich AS, Bozza FA. Platelets in dengue infection. Drug Discov Today Disease Mech 2011;8:33-8.
16. Schmidt AC. Response to dengue fever–The good, the bad, and the ugly. N Engl J Med 2010;363:484-7.
17. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: A review. J Nat Med 2013;67:677-89.
18. Ye J, Zhu B, Fu ZF, Chen H, Cao S. Immune evasion strategies of flaviviruses. Vaccine 2013;31:461-71.
19. Fink J, Gu F, Ling L, Tolfvenstam T, Olfat F, Chin KC, *et al.* Host gene expression profiling of dengue virus infection in cell lines and patients. PLoS Negl Trop Dis 2007;1:e86.
20. Whitehorn J, Farrar J. Dengue. Clin Med (Lond) 2011;11:483-7.
21. Murphy BR, Whitehead SS. Immune response to dengue virus and prospects for a vaccine. Annu Rev Immunol 2011;29:587-619.
22. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, *et al.* Dengue in the early febrile phase: Viremia and antibody responses. J Infect Dis 1997;176:322-30.
23. Chawla P, Yadav A, Chawla V. Clinical implications and treatment of dengue. Asian Pac J Trop Med 2014;7:169-78.
24. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, *et al.* Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373:1195-206.
25. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr 2004;79:727-47.
26. Bentz AB. A review of quercetin: Chemistry, antioxidant properties, and bioavailability. J Young Invest 2009;19:24-30.
27. Vikram V, Ramesh M, Prapulla S. Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. J Food Eng 2005;69:31-40.
28. Hullikere MM, Joshi CG, Vijay R, Mahesh M. Comparative analysis of papain from different varieties of Papaya plant latex. Int J Agric Food Sci 2014;4:123-7.
29. Priamo WL, Dalmolin I, Boschetto DL, Mezzomo N, Ferreira SR, de Oliveira JV. Micronization processes by supercritical fluid technologies: A short review on process design. Acta Sci Technol 2013;35:695-709.
30. Rababah TM, Al-u'datt M, Alhamad M, Al-Mahasneh M, Ereifej K, Andrade J, *et al.* Effects of drying process on total phenolics, antioxidant activity and flavonoid contents of common mediterranean herbs. Int J Agric Biol Eng 2015;8:145-50.
31. Wahala WM, Silva AM. The human antibody response to dengue virus infection. Viruses 2011;3:2374-95.
32. Valdés K, Alvarez M, Pupo M, Vázquez S, Rodríguez R, Guzmán MG. Human Dengue antibodies against structural and nonstructural proteins. Clin Diagn Lab Immunol 2000;7:856-7.
33. Available from: http://www.sciencenotes.ucsc.

edu/2012/pages/dengue/dengue.html. [Last accessed on 2015 Feb 21].
34. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, *et al.* Evaluation of diagnostic tests: Dengue. Nat Rev Microbiol 2010;8 12 Suppl: S30-8.
35. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MR, Rodrigues SG, *et al.* Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol 2006;13:1185-9.
36. Available from: http://www.cdc.gov/dengue/clinicalLab/laboratory.html. [Last accessed on 2015 Feb 25].
37. Aravind G, Debjit B, Duraivel S, Harish G. Traditional and medicinal uses of *Carica papaya.* J Med Plants Stud 2013;1:7-15.
38. Patil S, Shetty S, Bhide R, Narayanan S. Evaluation of platelet augmentation activity of *Carica papaya l*eaf aqueous extract in rats. J Pharmacogn Phytochem 2013;1:57-60.
39. Ayoola P, Adeyeye A. Phytochemical and nutrient evaluation of *Carica papaya*[pawpaw] leaves. IJRRAS 2010;5:325-8.
40. Arumugam N, Tualasinathan B, Rajarajeswari P, Duraimurugan M. Antimicrobial activity and phytochemical screening of *Cynodon dactylon* and *Carica papaya.* Res Biotechnol 2014;5:21-31.
41. Ranasinghe P, Ranasinghe P, Abeysekera WK, Premakumara GS, Perera YS, Gurugama P, *et al. In vitro* erythrocyte membrane stabilization properties of *Carica papaya* L. leaf extracts. Pharmacognosy Res 2012;4:196-202.
42. Anibijuwon I, Udeze A. Antimicrobial activity of *Carica papaya* (pawpaw leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria. Ethnobot LeaLf 2009;2009:850-64.
43. Afzan A, Abdullah NR, Halim SZ, Rashid BA, Semail RH, Abdullah N, *et al.* Repeated dose 28-days oral toxicity study of *Carica papaya* L. Leaf extract in sprague dawley rats. Molecules 2012;17:4326-42.
44. Canini A, Alesiani D, D'Arcangelo G, Tagliatesta P. Gas chromatography–mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J Food Composit Anal 2007;20:584-90.
45. Julianti T, De Mieri M, Zimmermann S, Ebrahimi SN, Kaiser M, Neuburger M, *et al.* HPLC-based activity profiling for antiplasmodial compounds in the traditional Indonesian medicinal plant *Carica papaya* L. J Ethnopharmacol 2014;155:426-34.
46. Anjum V, Ansari S, Naquvi KJ, Arora P, Ahmad A. Development of quality standards of *Carica papaya* Linn. leaves. Sch Res Lib 2013;5:370-6.
47. Kalil IC, Gibson BA, Ribeiro CA, Benincá, LA, Brasil GA, Andrade TU, *et al*. Antimutagenic activity of *Carica papaya* L. assayed *in vivo* by micronucleus test. Rev Ciên Farmacêuticas Básic Aplicada 2012;32:419-23.
48. Fadare O, Durosimi O, Fadare R, Izevbekhai O, Awonyemi IO, Obafemi CA. ATR-FTIR and HPLC spectroscopic studies and evaluation of mineral content of *Carica papaya* leaves and flowers. J Phytomed 2015;1:1-7.
49. Nakhoul IN, Kozuch P, Varma M. Management of adult idiopathic thrombocytopenic purpura. Clin Adv Hematol Oncol 2006;4:136-44, 153.
50. Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. J Ethnopharmacol 2010;127:760-7.
51. Panzarini E, Dwikat M, Mariano S, Vergallo C, Dini L. Administration Dependent Antioxidant Effect of *Carica papaya* seeds water extract. Evid Based Complement Alternat Med 2014;2014:281508.
52. Juárez-Rojop IE, A Tovilla-Zárate C, Aguilar-Domínguez DE, Lobato-García CE, Blé-Castillo JL, López-Meraz L, *et al*. Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. Rev Bras Farmacogn 2014;24:341-7.
53. Gamulle A, Ratnasooriya W, Jayakody J, Fernando C, Kanatiwela C, Udagama P. Thrombocytosis and anti-inflammatory properties and toxicological evaluation of *Carica papaya* mature leaf concentrate in a murine model. Int J Med Plants Res 2012;1:21-30.
54. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. Inflammopharmacology 2008;16:168-73.
55. Gupta A, Wambebe C, Parsons D. Central and cardiovascular effects of the alcoholic extract of the leaves of *Carica papaya*. Pharm Biol 1990;28:257-66.
56. Senthilvel P, Lavanya P, Kumar KM, Swetha R, Anitha P, Bag S, *et al.* Flavonoid from *Carica papaya* inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. Bioinformation 2013;9:889-95.
57. Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, Fazal L. Dengue fever treatment with *Carica papaya* leaves extracts. Asian Pac J Trop Biomed 2011;1:330-3.
58. Stanbridge EJ, Shafee N, Yusoff K. Inhibition of hypoxia-inducible factor activity with *Carica papaya* extracts. Google Patents; 2014.
59. Kaur G, Jalagadugula G, Mao G, Rao AK. RUNX1/core binding factor A2 regulates platelet 12-lipoxygenase gene (ALOX12): Studies in human RUNX1 haplodeficiency. Blood. 2010;115:3128-35.
60. Available from: http://www.rochway.com.au/papaya-leaf-extract-capsules-60-caps.html. [Last accessed on 2015 Dec 08].
61. Available from: http://www.herbalpapaya.com/collections/papaya-leaf-extracts/products/papaya-leaf-extract-capsules. [Last accessed on 2015 Dec 08].
62. Available from: http://www.herbalpapaya.com/collections/papaya-leaf-extracts/products/papaya-leaf-extract-1oz. [Last accessed on 2015 Dec 08].
63. Available from: http://www.sidomunculherbal.com/en/13-sido-muncul-sari-daun-pepaya.html. [Last accessed on 2015 Dec 08].

64. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: Importance and enhancement techniques. ISRN Pharm 2012;2012:195727.
65. Lane N. The evolution of oxidative stress. Principles of Free Radical Biomedicine. Vol. 1. Montreal, Quebec, Canada: Nova Science Publishers, Inc.; 2011. p. 1-18.
66. Catchpole OJ, Grey J, Mitchell K, Lan J. Supercritical antisolvent fractionation of propolis tincture. J Supercritical Fluids 2004;29:97-106.
67. Fages J, Lochard H, Letourneau JJ, Sauceau M, Rodier E. Particle generation for pharmaceutical applications using supercritical fluid technology. Powder Technol 2004;141:219-26.
68. Vandana KR, Raju YP, Chowdary VH, Sushma M, Vijay Kumar N. An overview on in situ micronization technique – An emerging novel concept in advanced drug delivery. Saudi Pharm J 2014;22:283-9.
69. Charles M. Fermentation scale-up: Problems and possibilities. Trends Biotechnol 1985;3:134-9.
70. Available from: http://www.nap.edu/read/1939/chapter/1. [Last accessed on 2015 Dec 10].
71. Singhania RR, Patel AK, Soccol CR, Pandey A. Recent advances in solid-state fermentation. Biochem Eng J 2009;44:13-8.
72. Firenzuoli F, Gori L. Herbal medicine today: Clinical and research issues. Evid Based Complement Alternat Med 2007;4 Suppl 1:37-40.
73. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem 2014;2:115-9.
74. Bimakr M, Rahman RA, Taip FS, Ganjloo A, Salleh LM, Selamat J, *et al*. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. Food Bioprod Process 2011;89:67-72.
75. Shah MV, Minal R. Novel techniques for isolation and extraction of phyto-constituents from herbal plants. Am J Phytomed Clin Ther 2013;1:2321-748.
76. Harbir K. Processing technologies for pharmaceutical tablets: A review. Int Res J Pharm 2012;3:20-3.
77. Sastry SV, Nyshadham JR, Fix JA. Recent technological advances in oral drug delivery - A review. Pharm Sci Technolo Today 2000;3:138-145.
78. Pushpalatha HB, Pramod K, Sundaram R, Shyam R. Pasteurization as a tool to control the bio-burden in solid herbal dosage forms: A pilot study of formulating Ashoka tablets with an industrial perspective. J Adv Pharm Technol Res 2014;5:191-5.
79. Available from: http://www.itmonline.org/arts/dosage.htm. [Last cited on 2015 Sep 11].

Source of Support: Nil. **Conflict of Interest:** None declared.