

The plausible mechanisms of action of *Carica papaya* on Dengue infection: A comprehensive review

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Abstract: Dengue is a serious health endemic with a prediction that 2.5 billion are at risk of acquiring dengue infection and 50 million are infected yearly. Over the years, tremendous effort has been done to develop potential treatment for dengue infection. However, to no avail, most of the treatment modalities have failed in clinical evaluation and trials. With the lack of treatment for dengue infection, home remedies have been widely used as a belief to relieve the symptoms, one of them is the use of papaya leaf juice. This review provides a recent evidences and research that have been attempted to unravel the mechanism of actions of papaya leaf extract to treat dengue infection. Up to date, there are three main mechanisms that have been proposed, which are the anti-thrombocytopenic, immunomodulatory and anti-viral effect of papaya leaf juice extract.

Keywords: *Carica papaya*; dengue; *Aedes ayegypti*; platelet; mechanism

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Introduction

Dengue fever is a most prevalent infection caused by the Flavivirus (family: Flaviviridae) that transmitted by the female vector mosquito *Aedes ayegypti*^[1]. One of the main regions that are infected with the dengue are the America, Africa and Asia countries such as Malaysia, Philippines, Singapore, Sri Lanka and Thailand, with an estimated mortality of 9 per million populations reported in 2013^[2] (Figure 1). As a global endemic, it is predicted that 2.5 billion are at risk of acquiring dengue infection and 50 million are infected yearly^[3]. Dengue infections can be caused by 4 serotypes of virus which are DEN-1, DEN-2, DEN-3 and DEN-4^[4]. DENV-1 was first reported in French Polynesia and Japan, and constantly increased over time in Asian regions. Meanwhile DENV-2 was first reported in Indonesia and Papua New Guinea and spread thru Asian regions such as Malaysia,

Thailand, the Philippines and Singapore since then. Both DENV-3 and DENV-4 were reported much later in 1953 in the Philippines and Thailand^[5].

Studies have been done to figure out the relationship between the dengue serotypes and their respective clinical manifestations. Infection with DEN-1 and DEN-3 has mild clinical manifestations, whereas infection cases with DENV-2 showed severe manifestations amongst all^[5]. The infection varies from asymptomatic illness to severe ones such as haemorrhagic fever and dengue shock syndrome. The clinical manifestations of dengue infections are thrombocytopenia, leukopenia, and transient neutrophil deficiency with coagulation abnormalities^[6]. It is suggested that those manifestations are due to the abilities of dengue virus in inhibition of the megakaryocytes differentiation or activation of platelets^[7].

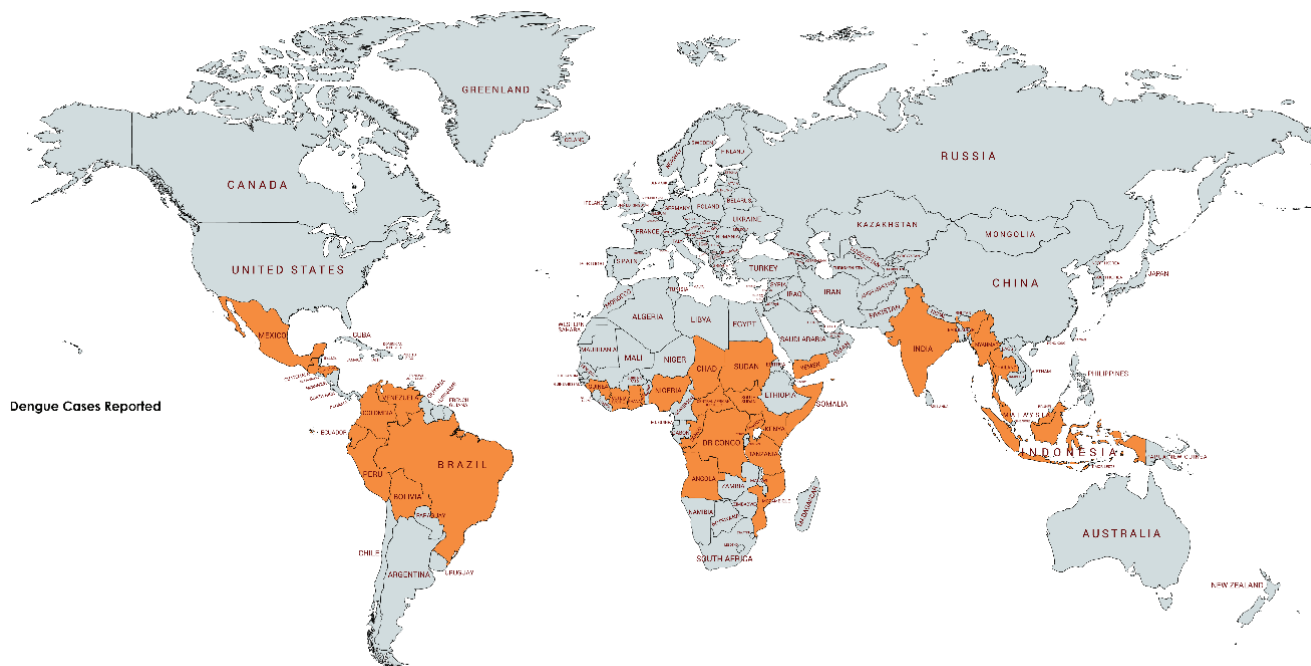


Figure 1. Geographical distribution of dengue infection worldwide^[2].

Treatment for Dengue Infection

As an endemic, dengue infection has caused a substantial impact on the health, social and economy globally. The estimates of economic burden of dengue are roughly 40,000 disability-adjusted life years (DALYs) annually, which inflicted a loss of US\$ 94.87 million per year as in 2016^[8]. This figure has pursued the urgency to seek for prevention and treatment of dengue. Over the years, tremendous effort has been done to develop potential treatment modalities such as corticosteroids, immunoglobulin, host immune modulator and anti-viral agents^[9-11]. However, to no avail, the mentioned modalities have failed in clinical evaluation and trials^[11]. To date, the main clinical managements of dengue infection are the supportive measures such as anti-pyretic, hydration, blood transfusion and pain killers^[12]. Similar to other viral infection, the dengue infection induces a life-long immunity to specific serotypes. Nevertheless, the conditions of dengue infection will be life-threatening for second infection from another serotype^[13]. Currently, the only preventive measure for this infection is by preventing the bite from the mosquitoes with virus vector and by using the insecticides and larvicides which is potentially hazardous to environment and human beings.

With the lack of treatment for dengue infection, home remedies have been widely used as a belief to relieve the symptoms. In South East Asia, most of the dengue patients rely on numerous home remedies for managing dengue infection such as isotonic drinks, crab soups and papaya leaves^[14]. Papaya leaf juice has been widely used by traditional healers in Asian regions such as North Eastern of India, Goa, Uttar Pradesh and Odisha in

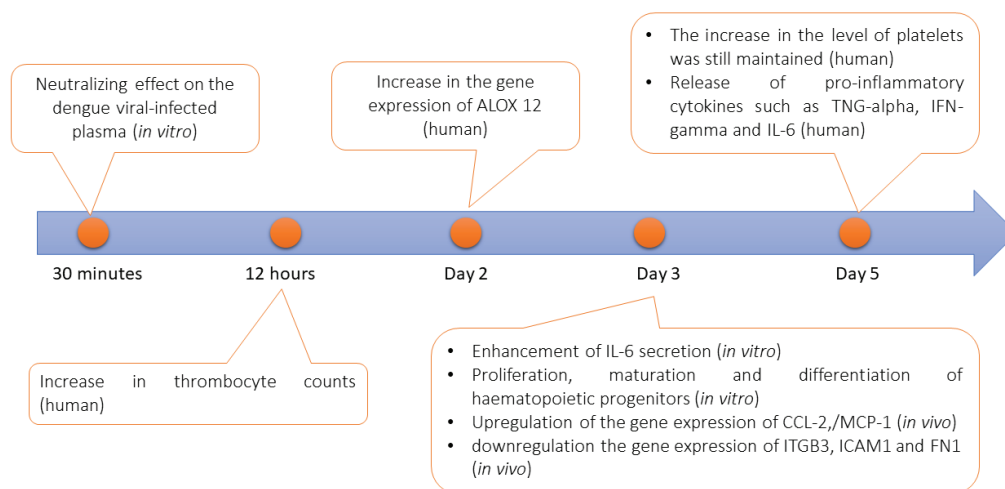
dengue patients^[15]; for instance, In Thailand, papaya leaf juice is so popular during monsoon season that a delivery guy will bottle and deliver the juices all over the towns^[16]. Following the popular use of papaya leaf for the treatment of dengue, research have been done to evaluate the potential of papaya leaves and its underlying mechanisms of action.

Carica papaya

Carica papaya Linn., which is commonly known as papaya or pawpaw, is the palm like tree that is the only species within the Caricaceae genus. The tree produces segmented yellow flowers and large black seeded yellow to orange fruits^[17]. As a popular home-growing crops in tropical regions, the papaya has been used for centuries as a ethnomedicine to treat many diseases includes dengue fever^[18]. The leaves were found to be rich in phenolic compounds such as alkaloids, quercetin and kaempferol. On top of that, the leaf extract contains antioxidant compounds such as α -tocopherol, ascorbic acid and flavonoids^[19-21]. Some case studies reported that the papaya leaves aid for better recovery of dengue in terms of clinical symptoms and platelet counts. With that, more research have been performed in recent years to elucidate the plausible mechanisms of action of papaya on dengue. From the literature review, it is suggested that the beneficial effect of papaya leaf could be exerted thru three mechanisms of action, which are the recovery of platelet counts, inhibition of pro-inflammatory cytokines and larvicidal effect (Table 1). As presented in Figure 2, this review has also compiled the timeline of the effect of papaya leaf extract, demonstrating the suitable or suggestive period of administration of papaya leaf extract for dengue treatment.

Table 1. The plausible mechanisms of action of papaya on dengue treatment.

Authors	Study	Study Characteristics	Mechanisms
Anti-thrombocytopenic Effect			
Sathiasivam <i>et al.</i> (2009)	<i>In vivo</i> (Swiss albino mice)	Oral administration of 15 mg/kg of powdered papaya leaves in palm oil	· increase in the platelet count observed within 72 hours
Anjum <i>et al.</i> (2017)	<i>In vivo</i> (Wistar rat)	Oral administration of 150 mg/kg of aqueous extract of papaya leaf	· significant increase in the thrombocyte counts temporarily up to 12 hours
Siddique <i>et al.</i> (2014)	Human	Oral administration of 150 mL of papaya leaf juice for 5 days	· significant increase in the thrombocyte counts · exhibit anti-hemolytic effect
Sathyapalan <i>et al.</i> (2020)	Human	Oral administration of 1100 mg of papaya leaf extract in tablet three times a day for 5 days	· increase in the platelet count in the 1st 4 days and maintained on day 5
Subenthiran <i>et al.</i> (2013)	Human	Oral administration of 50 mg of papaya leaf juice extract once a day for 3 days	· Significant increase in the platelet counts at 40 and 48 hours · 15-fold increase in the gene expression of ALOX 12
Immunomodulatory Effect			
Sathyapalan <i>et al.</i> (2020)	Human	Oral administration of 1100 mg of papaya leaf extract in tablet three times a day for 5 days	· initiation and activation of the coagulation and inflammation pathways lead to the release of pro-inflammatory cytokines such as TNG-alpha, IFN-gamma and IL-6
Aziz <i>et al.</i> (2015)	<i>In vitro</i> (Mesenchymal stem cells and haematopoietic cells)	Treatment of unripe papaya leaf juice extract (10% v/v) for 72 hours	· Enhancement of IL-6 secretion and lead to the proliferation, maturation and differentiation of multipotential haematopoietic progenitors · production of thrombopoietin in the liver triggered by IL-6
Razak <i>et al.</i> (2019)	<i>In vivo</i> (AG129 mice with non-mouse adapted dengue virus)	Oral administration of 1000 mg/kg freeze-dried papaya leaf juice for three days	· Upregulation of the gene expression of CCL-2/MCP-1 · downregulation the gene expression of ITGB3, ICAM1 and FN1
Anti-viral Effect			
Chinnappan <i>et al.</i> (2016)	<i>In vitro</i> (blood samples from dengue patients)	Freeze-dried papaya leaf juice for 30 minutes (dose is not mentioned)	· neutralizing the dengue viral-infected plasma
Sathyapalan <i>et al.</i> (2020)	Human	Oral administration of 1100 mg of papaya leaf extract in tablet three times a day for 5 days	· significant decrease in the NS1 viraemia level
Senthivel <i>et al.</i> (2013)	<i>Ex vivo</i> (Bioinformatics tool)	Docking of quercetin onto the catalytic site of the serine protease enzyme NS2B-NS3 is performed using SYBYL — Surflex docking	· Inhibition of NS2B-NS3 protease · Prevention of Dengue 2 Viral assembly

**Figure 2.** Timeline of the effect of papaya leaf extract.

Anti-thrombocytopenic Effect of *Carica papaya*

Based on the current understanding on the ability of *Carica papaya* extract to increase the platelet count, there is plausible explanation that it may be related to the stimulation of the thrombopoietic mechanism. In an *in vivo* study carried by Anjum *et al.* (2017), there was an increase in the platelet count observed in the Wistar rat within 72 hours after the oral administration of 150 mg/kg of aqueous extract of papaya leaf. The study has postulated that the papaya leaf aqueous extract can stimulate and induce the megakaryopoietic/thrombopoietic

stimulatory activity or cause splenic contraction to produce thrombocytes. However, the detailed downstream mechanisms were not further described and revealed in the study. On top of that, it is suggested that the increase in platelet activity might due to the presence of high flavonoids and phenolic compounds such as kaempferol, trans-ferulic acid, caffeic acid and myricetin in the papaya leaf extract^[22]. Another study carried out by Sathiasivam *et al.* (2009) claimed that the oral administration of 15 mg/kg of powdered papaya leaves in palm oil has significantly increased the thrombocyte counts temporarily up to 12 hours in Swiss albino mice, indicating that

papaya extract that was dissolved in oil with a lower dose (15 mg/kg) could also exert the anti-thrombocytopenic effect. As the effect was transient up to a short period of time, the mechanism of action has not been determined as well^[23].

Several case studies have been carried out in dengue patients to investigate the effect of papaya leaf extract. Siddique *et al.* (2014) proved that the oral consumption of papaya leaves juice has significantly increase the thrombocyte counts as compared to control groups within 5 consecutive days in dengue patients. Apart from increase in the thrombocyte, the study also suggested the improvement of the symptoms in dengue might due to the anti-hemolytic activity of papaya too^[12]. In another study by Sathyapalan *et al.* (2020), the patients with severe thrombocytopenia who has taken 1100 mg of papaya leaf extract in tablet three times a day for 5 days showed an increase in the platelet count in the first 4 days and then the platelet count is maintained on day 5 compared to the placebo group in which the platelet count increases slowly and the platelet count drops on day 5. This indicates that the *Carica papaya* leaf extract has the capability to maintain the platelet count where it promotes the production of platelets and could be used in severe condition, thus proving the reliability as an alternative treatment for dengue patients^[24].

This anti-thrombocytopenic effect is also supported by Subenthiran *et al.* (2013) in which they discovered that the gene in the red blood cells known as ALOX 12 has increased by 15-fold among dengue patients who took *Carica papaya* leaf juice (50 grams) for three days. This ALOX 12 gene is known to increase the megakaryocyte production where it will lead to the conversion to platelets, thus increasing the platelet production. The platelet increase was significant at 40 hours and 48 hours, suggesting that the papaya leaf extract could rapidly induce the production of platelets in human^[25].

In a systematic review conducted by Charan *et al.* (2016), four different formulations of *Carica papaya* extract which are leaf juice, capsule form, syrup form and tablet form were given to dengue patients. Interestingly, the results showed that the platelet count has significantly increase ($p=0.005$) in all formulations as compared to the control group. Henceforth, regardless of different formulation, this suggests that *Carica papaya* extract has a good bioavailability where it can accommodate high compliance in different populations that are having other health issues such as those with difficulty in swallowing or children^[26]. Another systematic review by Rajapakse (2019) critically analysed that even the dose use of papaya leaf extract are varies, no effective dose have been determine, the platelet counts of patients is increasing at day 3–5 following papaya juice, substantially shortening the hospital stay of the patients^[2].

Immunomodulatory Effect of *Carica papaya*

Research have been done to investigate the beneficial effect of papaya leaf extract as an immune-boosting impact to the dengue patients. A study by Sathyapalan *et al.* (2020) proposed that oral administration of 1100 mg of papaya leaf extract in tablet three times a day for 5

days in human has initiated and activated the coagulation and inflammation pathways lead to the release of pro-inflammatory cytokines such as TNG-alpha, IFN-gamma and IL-6, that further inhibit the thrombocytopenia in patients^[24]. Similarly, another research team has proposed that the unripe papaya leaf juice extract (10% v/v) which consists of major compound papain, may enhance IL-6 secretion and lead to the proliferation, maturation and differentiation of multipotential hematopoietic progenitors and then increase in thrombocyte production in mesenchymal stem cells and haematopoietic cells. Interestingly, the study also proposed that the increase in thrombocyte can be due to the action of production of thrombopoietin in the liver triggered by IL-6^[27]. An *in vitro* study carried out by Razak *et al.* (2019) proved that the 1000mg/kg of oral administration of freeze-dried *Carica papaya* leaf juice for three days has upregulated the gene expression of CCL-2/MCP-1 and downregulated the gene expression of ITGB3, ICAM1 and FN1 in AG129 mice with non-mouse adapted dengue virus, suggesting the papaya juice has hindered the endothelial permeability and resulted in the inhibition of cytokines expression from immune cells^[28].

Anti-viral Effect of *Carica papaya*

Carica papaya extract has not only proven to increase the platelet count, but also exhibits antiviral activity. Another mechanism of action of papaya on dengue was proposed by Chinnappan *et al.* (2016), in which the papaya extract exerts its action on the platelet directly, by preventing the activation and aggregation of platelets and also neutralizing the dengue viral-infected plasma^[29]. In a study conducted by Sathyapalan *et al.* (2020), they discovered that there was a significant decrease in the NS1 viraemia level ($p=0.023$) at Day 3 in the patient group that are taking 1100 mg of papaya leaf extract. *Carica papaya* leaf extract may have inhibitory activity against NS2B-NS3 protease, thus preventing dengue 2 viral assembly^[30]. The research team has discovered that quercetin, one of the major components in the papaya leafextract, binds to the NS2B-NS3 protease which are important in viral replication. It is proposed that quercetin has a better stability and ligand enzyme interaction as they form 6 hydrogen bonds with the amino acid at the binding site of the receptor as compared to other compounds such as caffeic acid only form 4 hydrogen bonds which is lesser than the quercetin. The research has strongly suggested that the quercetin could be developed as an effective preventive compound for dengue virus infection^[30].

Conclusion and Future Perspective

The review is intended to provide an overview of the recent outcomes of the papaya on combating dengue infection with emphasis on the targeting mechanisms of action (Figure 3).

To date, around 2168 patents have been granted for the health potential of the aerial parts of *Carica papaya* (www.lens.org). It is reported the total cost for a dengue fever patient was around USD 141–385 in South East Asia^[31] and accounts for 0.03% of the GDP per capita in South East Asia countries in 2010^[32]. In view of the widely availability of papaya leaves, it is predicted that the ten-

tative cost of treatment using papaya leaves will be lowered as compared with conventional treatment provided. As noted in the review, in spite of the sufficient evidences from pre-clinical *in vitro*, *in vivo*, *ex vivo* and small scale clinical trial in dengue patients, the papaya juice extract has not been translated into an approved clinical modality for dengue infection. It is suggested that a large scale of randomised placebo controlled trial should be conducted to further evaluate the potential of papaya leaf extract as dengue as researchers and clinicians are still in doubt of the role of papaya leaves based on few positive preliminary results, limited toxicity profile and unclear dosing regimens. In a nut shell, there is a need to have high quality clinical trials to provide scientific evidence to dispel the myth on using a papaya leaf extract in treating and prevention of dengue infection.

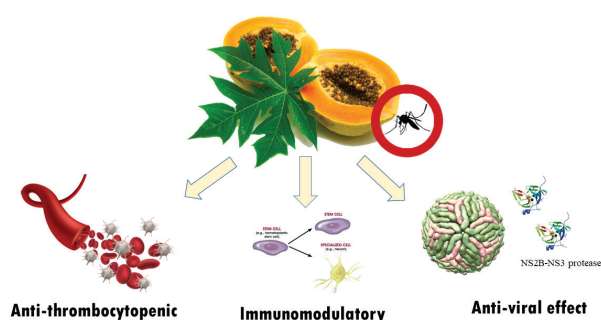


Figure 3. Possible mechanisms of *Carica papaya* leaf juice on dengue infection.

Author Contributions

ZKB, MB, YXJ and YRK participated in the writing of the manuscript. YSO and KKY conceptualized the project.

Conflict of interest

The authors hereby declare no competing interest.

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