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Assessing the Efficacy of *Carica papaya* Leaf Extract in Preventing Thrombocytopenia: A Review

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Abstract

A clinical condition known as thrombocytopenia is characterized by a low platelet count (less than 150 × 103/µl) in the blood, which leads to an imbalance in hemostasis and can result in multiple deadly consequences. Although the exact causes are not always known, they always lead to death by interfering with the formation of platelets and encouraging their destruction. Research demonstrated that Carica papaya leaf has special medicinal and therapeutic properties that help prevent thrombocytopenia. By promoting platelet formation, preventing platelet breakdown, and stopping viral proteases, secondary metabolites and minerals found in leaves such as carpaine and quercetin, respectively, support platelet membrane integrity. Examining research conducted on animals and in clinical trials, the present review investigated the scientific evidence supporting the protective effects of papaya leaf juice, extract, or powder against thrombocytopenia. Flavonoids, alkaloids, phenols, cardiac glycosides, tannins, terpenes, and saponins were found in the phytochemical profiles of Carica papaya leaves, which give the leaf potential for medicinal use. Antiviral, antidiabetic, anticancer, antimalarial, antiangiogenic, anti-oxidant, and immunomodulatory properties are among the leaf's medicinal advantages. A scientific study has demonstrated the effectiveness of Carica papaya leaf against thrombocytopenia in multiple trials, extending the use of natural sources to treat a wide range of illnesses.

Keywords: thrombocytopenia, immunomodulatory, anti-angiogenic, *Carica papaya*, antibacterial activity

INTRODUCTION

Throughout history, people have used plants and plant-derived products to prevent sickness; now, almost 80% of the world's population gets their primary healthcare from plants [1]. Given its great botanical diversity, India, in particular, is home to an estimated 45,000 plant species having therapeutic qualities [2]. Natural chemicals derived from plants have several advantages over synthetic medications, such as affordability, ease of use, and low side effects. These home cures have long been a mainstay of conventional medical systems, and researchers are still looking at how they may be used therapeutically in contemporary healthcare [3]. Numerous scholarly articles have documented the use of therapeutic herbs in the management of an extensive array of ailments. *Carica papaya* Linn.—a member of the family Caricaceae—is indigenous to the southern parts of Mexico and Central America. This plant, which is widely grown in India, is valued for its therapeutic properties all over the world [4]. The papaya plant can grow up to 20 m tall and is distinguished by its single smooth stem, large leaves with 5–6 lobes, and perennial nature [5]. Throughout the world, different components of the papaya plant, including the fruit, bark, roots, seeds, peel, pulp, and leaves, are used for different medicinal purposes [6, 7]. Papain is a highly abundant enzyme

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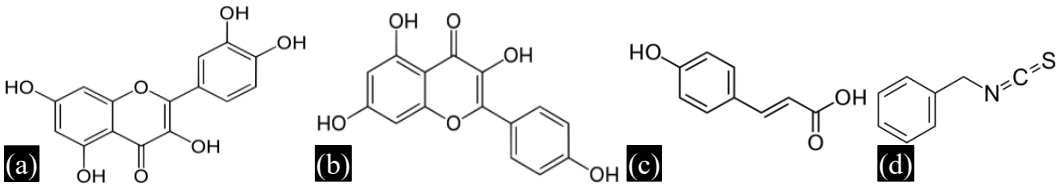

Figure 1. Chemical constituents present in *Carica papaya*: (a) Quercetin; (b) Kaempferolp; (c) p-Coumaric acid; and (d) Benzyl isothiocyanate.

found in papayas that is used to cure ulcers and aid in digestion. Additionally, it demonstrates particular efficacy against Gram-negative bacteria in microbiological infections. Higher amounts of benzyl isothiocyanate (BITC), which has bacteriostatic, fungicidal, and bactericidal properties, are present in the papaya fruit seed extract. About 4–5 g of seeds, or 25–30 mg of BITC, make up an effective dosage [8]. Numerous scientific investigations into the medical uses of papaya leaf have been conducted recently. Papaya leaves have long been used to cure a variety of illnesses, including fever, asthma, colic, beriberi, and jaundice [9]. The papaya plant, known for its nutritional richness in vitamins A, B, and C, along with calcium and iron, [10] showcases potent antioxidant properties, mitigating free radical activity and preventing disease onset [11].

Enzymes such as papain, glycyl endopeptidase, chymopapain, and caricain are scattered throughout the plant sections of papaya latex, which is an important component [12]. Papaya leaf extract (PLE) has been extracted using a variety of techniques, such as aqueous, ethanol, methanol, and freeze-dried papaya leaf juice, with the goal of preventing disease [13, 14]. The leaf of the papaya plant is a good nutritional supplement since it is high in proteins, fats, vitamins, and carbohydrates. Ascorbic acid stands at 38.6%, protein at 5.6%, phosphoric acid at 0.225%, carbohydrates at 8.3%, iron at 0.0064%, and minerals like magnesium at 0.035% per 100 g of leaf part. These numbers are representative of the various amounts of these constituents [15, 16].

According to quantitative phytochemical analysis, aqueous PLE contains 0.001% tannins, 0.022% saponins, 0.013% flavonoids, 0.011% phenolics, 0.019% alkaloids, and 0.004% steroids [17]. PLE offers a therapeutic option for preventing dengue—a viral disease [18]. Recent research by Otsuki and colleagues has highlighted PLE's impact on inhibiting tumor cell growth and increasing the secretion of TH1 type cytokines from human lymphocytes [19]. Moreover, papaya leaf contains several active constituents such as ascorbic acid, alpha-tocopherol, chymopapain, cyanogenic glucosides, cystatin, flavonoids, glucosinolates, and papain, which enhance antioxidant power in the blood and reduce lipid peroxidation levels [20].

The structures of bioactive secondary metabolites derived from PLE were depicted in Figure 1. There are a lot of anecdotal references available about the treatment of different malignancies, including lung adenocarcinoma, osteosarcoma, hepatocellular carcinoma, pancreatic epithelioid carcinoma, cervical carcinoma, mesothelioma, and breast cancer [21–23]. Furthermore, research on the anticancer qualities of PLE made by Australian Aboriginals has been recorded [24]. The goal of several scientific studies has been to separate and identify the bioactive components of papaya leaves. According to photochemical tests, young leaves have antibacterial, anti-inflammatory, antiviral, hypoglycemic, and anticancer activities because they include alkaloids, saponins, tannins, flavonoids, and glycosides [25]. The present review primarily highlighted the therapeutic properties of *C. papaya* leaf in preventing and managing the progression of diseases.

BIOLOGICAL SOURCE OF PAPAYA LEAF

The papaya plant, scientifically known as *Carica papaya*, is the biological source of papaya leaves. Papayas are succulent fruits that are produced by huge Caricaceae plants. Papaya is thought to be the offspring of the union of two or more Mexican and Central American *Carica* species, while its precise origins are still unknown. Papaya trees grow around the world, especially in Laie, Hawaii [26] (Figure 2).

Figure 2. *Carica papaya* plant

Chemical Constituent of Papaya Leaf

Sigma-Aldrich (St. Louis, Missouri, USA) provided rutin hydrate (HPLC grade; purity >94%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Merck (Darmstadt, Germany) provided formic acid (98–100%), acetonitrile (HPLC grade), methanol (HPLC grade), hexane (analytical grade), and ethanol (analytical grade). Trypsin, Penicillin/Streptomycin, high-glucose Dulbecco's Modified Eagle's Medium (DMEM), and fetal bovine serum (FBS) were obtained from Invitrogen (Life Technologies, Mulgrave, VIC, Australia). The American Type Culture Collection provided the human skin cell line (Hs27 ATCC CRL-1634) (Manassas, VA, USA) [27].

THERAPEUTIC APPLICATION OF PAPAYA LEAF

PLE is recognized for its medicinal properties in treating a variety of human diseases due to its abundance of phytochemicals, minerals, and vitamins. Numerous studies have documented the efficacy of PLE in managing various diseases since ancient times. Moreover, recent scientific investigations have highlighted the potential of papaya leaf in disease prevention, as summarized below [28].

Mechanism of Action of Papaya Leaf in Health Management

PLE (Pycnogenol), extracted from French maritime pine bark, exhibits a wide range of interactions with molecular targets, contributing to its therapeutic effectiveness against various diseases, including cancer prevention. Key mechanisms involved in its anticancer activity include the inhibition of DNA topoisomerase I/II, modulation of signaling pathways crucial for cancer cell survival, downregulation of anti-apoptotic Bcl-2 and Bcl-XL proteins, upregulation of pro-apoptotic Bax, Bak, and cleaved caspase 3, as well as enhancement of p53 expression. These actions collectively promote apoptosis and inhibit cancer progression, highlighting PLE's potential as a valuable agent in cancer therapy [29, 30].

Treatment with polymyallenium (PLE) has been shown to increase NO generation and increase the expression of TNF-α, CD80, IL-12p40, IL-6, IL-12p70, and IFN-γ costimulatory receptors, while decreasing IL-2 and IL-4 secretion [30]. Additionally, PLE influences the secretion of pro-inflammatory cytokines such as IL-1β, IL-6, IL-1α, IL-8, and chemokines such as CCL7, CCL2, and CCL8 [31].

Furthermore, PLE treatment has been reported to be beneficial for dengue patients by activating the expression of *ALOX 12* and *PTAFR* genes [32]. Dehydrated in a freezer in dengue virus-infected mice, *C. papaya* leaf has also been shown to modulate the production of inflammatory cytokines, such as CCL6/MRP-1, CCL17/TARC, CCL12/MCP-5, CCL8/MCP-2, IL1RN/IL1Ra, IL1R1, PF4/CXCL4, and NAMPT/PBEF1 [33]. Moreover, PLE has been shown to regulate beta cells for insulin release in diabetic rats [34].

Anti-dengue Effect of Papaya Leaf

A major worldwide health hazard—dengue —affects about 50–100 million people per year on an average. It is brought on by the dengue virus (DENV) types 1–4, which belongs to the Flaviviridae family and are spread via mosquito bites from *Aedes aegypti* [35]. The dengue virus usually takes 5–7

days to incubate before causing symptoms, which include vomiting, headaches, rash outbreaks, high fevers, and muscle aches [36]. One of the most important signs of dengue is thrombocytopenia, which is defined by a drop in platelet count [37]. Thrombocytopenia is defined by the World Health Organization (WHO) as a rapid decrease in platelet count, as evidenced by a platelet count of less than 150,000 per microliter of blood [38]. There are no antiviral drugs or vaccinations available right now to treat dengue fever. In order to prevent or treat the condition, patients usually require supportive care, which includes blood transfusions, blood components, and fluids. Nonetheless, there is still hope for the creation of a dengue vaccine thanks to current clinical trials. Investigating different strategies is essential to solving dengue's problems. Numerous studies have been conducted on herbal medicine as a possible alternative treatment for dengue complications. The potential of PLE, in treating dengue-related thrombocytopenia has been the subject of recent research. According to these studies, PLE treatment considerably raises platelet counts, which are frequently low during dengue illness [39].

One investigation revealed a rapid rise in platelet count among five dengue patients within a 24-hour period following PLE treatment [40]. Similarly, a randomized controlled trial involving 285 patients found that papaya leaf syrup increased the average platelet count as compared to controls [41]. Consistent results were observed in a study involving 228 patients in Malaysia [42]. Research suggested that decreased platelet counts, along with increased vascular permeability and plasma leakage, are primary bleeding factors in dengue patients [43] PLE possesses membrane-stabilizing properties that shield blood cells from stress-induced destruction, potentially preventing platelet lysis in dengue patients [44].

Furthermore, in dengue virus-infected mice, the production of inflammatory cytokines, such as CCL12/MCP-5, CCL8/MCP-2, CCL17/TARC, CCL6/MRP-1, IL1RN/IL1Ra, IL1R1, PF4/CXCL4, and NAMPT/PBEF1, was markedly decreased by freeze-dried *C. papaya* leaf juice [45]. Currently, PLE has been shown to diminish the expression of DENV NS1 envelope protein and stimulate the upregulation of IFN-α expression in THP-1 cells. These findings are promising and underscore the need for comprehensive investigations involving animal models and clinical trials to establish the standardized use of PLE for preventing and treating dengue infections [46].

Immunomodulatory Effect of Papaya Leaf

Experiments on various portions of the papaya plant have shown that it has important medicinal characteristics that are helpful in treating a variety of pathological illnesses, including cancer, dengue fever, cardiovascular disease, and wound healing [47]. Strong immunomodulatory, anticancer, and anti-inflammatory effects have recently been demonstrated by PLE in a variety of cancer cell lines [48]. On the other hand, reports on peripheral blood mononuclear cells (PBMC) are scarce.

PLE's immunomodulatory potential was investigated, and the cytokine profile of human PBMC was examined using ELISA in a 2010 study. According to the results, PLE dose-dependently reduces the secretion of IL-4 and IL-2 in culture supernatants, which may imply that PLE induces apoptosis in PBMC in a manner like to that which works on cancer cells. Remarkably, at modest doses of PLE, secretion of Th1-type cytokines such as IL-12p70, IL-12p40, TNF-α, or IFN-γ, which are relevant to anti-tumor immunity, was elevated with little effect on IL15, IL-6, IL-5, and IL-10 production.

These findings implied that PLE could improve the treatment of Th2-mediated allergic diseases such as allergic rhinitis and bronchial asthma, or function as an adjuvant for different vaccines by encouraging a transition of the immune system from Th2 to Th1 type [49].

The findings indicated a noteworthy correlation between elevated Th1-type cytokine secretion and heightened cytotoxicity, given that IFN-γ, TNF-α, and IL-12 are strong inducers of cell-mediated cytotoxicity [50]; perhaps resulting in enhanced protection against tumors [51]. Various in vitro studies have highlighted the role of bioactive compounds found in PLE, particularly those extracted using polar solvents such as alcohol and water, in modulating immunoinflammatory markers [52].

Bertrand et al. showed that the bacterial endotoxin lipopolysaccharide (LPS) stimulates innate immunity by influencing the production of many inflammatory mediators in monocytes/macrophages, including IL-6, IL-1β, IFN-γ, and TNF-α [53]. Additionally, it was found that in LPS-stimulated human PBMCs, the methanol extract of papaya leaf decreased the secretion of pro-inflammatory cytokines IL-6, IL-1β, IL-1α, TNF-α, and IL-8 by 42.9%, 27.4%, 12.5%, 10.8%, and 8.4%, respectively [54]. Moreover, in 2011, a reduction in nitric oxide (NO) secretion was reported in IFN-γ or LPS-activated murine macrophage cells (RAW 264.7 cell line) treated with methanolic PLE [55].

According to a study, malondialdehyde, glutathione, catalase, and superoxide dismutase levels can all be controlled by an aqueous extract of unripe papaya fruit. Furthermore, it notably increased the levels of immunoglobulins IgG and IgM in rats treated with acrylamide [56]. In summary, the in vitro findings discussed indicated that papaya extracts possess the potential to control the expression of inflammatory markers in various stressed cell types. Although polar solvents have been used extensively in the in vitro studies for papaya extracts; further research is necessary to fully comprehend the anti-inflammatory properties of nonpolar extracts. Animal experiments are required to evaluate the biological effects and possible uses of plant extracts in preclinical settings. Eating papaya in different forms—fruit, peel, and leaf—affects antioxidant enzymes, platelet counts, and inflammatory indicators in both people and animals. Additional in vivo research on humans and animals has revealed that PLE has anti-inflammatory and platelet-increasing qualities. Animal tests have been conducted to investigate the immunomodulatory action and mechanisms of papaya fruit, despite the paucity of in vitro studies on the subject [55].

Antibacterial Activity of Papaya Leaf

Several studies have highlighted the antibacterial properties of *C. papaya* leaf extracts. In one study, Suresh et al. found that among five plant extracts tested, *C. papaya* leaf extract displayed the most potent antibacterial activity [57]. Specifically, it effectively inhibited the growth of Gram-positive bacteria such as *Pseudomonas aeruginosa, Bacillus subtilis*, and *Staphylococcus aureus*, while showing limited impact on Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* [58]. The robust outer membrane of Gram-negative bacteria, characterized by a thick murein layer, restricts the entry of inhibitory substances from plant extracts into the cell.

Significant bactericidal activity against *B. cereus, K. pneumoniae, Micrococcus luteus, E. coli, P. aeruginosa,* and *S. aureus* was demonstrated by papaya leaves extracted using different solvents (ethanol, methanol, ethyl acetate, acetone, chloroform, or hot water) in another investigation. Furthermore, *S. aureus, E. coli,* and *Candida albicans* were also susceptible to the antibacterial properties of the PLE methanolic extract [59]. These studies represented initial inquiries, prompting further exploration into the molecular mechanisms underlying the antibacterial properties of *C. papaya* leaf extracts [60].

COLLECTION OF PAPAYA LEAVES

The procedure began with the separation of blanched (pre-treatment) and non-blanched (control) papaya leaves, which were then arranged in aluminum trays for each drying method. Three drying techniques were utilized that is, oven drying, involving temperatures of 40°C for 7 h, 50°C for 6 h, 60°C for 4 h, and 120°C for 15 min [61]. Fresh papaya leaves sourced from Kota Kinabalu underwent a washing process under running tap water followed by sterile distilled water. Subsequently, the leaves were air-dried at 40°C for 72 h, [62] and then finely ground into powder using a mechanical blender (Waring® Commercial Blender). After calculating the dry weight, the powder was kept at -20°C in an airtight container until needed [63].

Solvent Extraction of Papaya Leaf

The compounds present in papaya leaves were extracted using polar and non-polar solvents, namely methanol, acetone, chloroform, ethyl acetate, and n-hexane. About 100 g of powdered leaves were submerged in conical flasks containing 1000 ml of each solvent. These flasks underwent sonication

(Branson® 5510) for 10 min at 25°C. The resulting extracts were then filtered using Whatman No. 1 filter paper and evaporated using a Rota Vapor (BUCHI) at 40°C, 150 rpm, until a final volume of 1 ml of extract per 10 g of plant sample was obtained. The aliquots were weighed and stored at 20°C for future use. The extraction yield was calculated using the given formula:

$$\text{Yield } (\%) = \frac{\text{Dry weight of extract}}{\text{Dry weight of powder}} \times 100$$

In Vitro Antimicrobial Assay

Crude extracts were dissolved in Dimethyl sulfoxide (DMSO), and serial concentrations of plant solvent extracts at 5 mg ml^{-1}, 15 mg ml^{-1}, 30 mg ml^{-1}, and 45 mg ml^{-1} were prepared. Agar with DMSO (without plant extract) served as the negative control, while agar without DMSO or extract was the absolute control. *Ganoderma boninense* was extracted from the edge of a seven-day-old culture using a sterile micropipette tip (0.8 cm) and placed in the middle of the media. Pathogen growth was measured (in cm), with each extract concentration assayed in triplicate and mean values calculated. The ED50 for both methanol and acetone crude extracts was determined using SPSS Probity analysis [64].

CONCLUSION

The present study concluded that PLE had potent antimicrobial, antiviral, anticancer, hypoglycemic, and anti-inflammatory effects. Additionally, in order to investigate papaya leaf's medicinal potential, clinical trials are required. Traditional medicine has traditionally utilized *C. papaya* (papaya) leaf extract to treat fever in a number of infectious disorders, including dengue, malaria, and chikungunya. The advancement of science and technology has consequently made it feasible to present proof that this plant is useful not only as a folk remedy but also has pharmacological and toxicological properties that have been scientifically demonstrated. These properties have led to the plant's official use in formal healthcare systems. This product is currently more valuable due to the development of formulations for use in cosmeceuticals and nutraceuticals. The use of good manufacturing practice (GMP) standards and the national government's regulations making product registration easier will undoubtedly raise the value of PLE in the near future as a crucial nutraceutical and cosmeceutical product. In light of its prospective health advantages and industrial uses, PLE is reviewed in the present article as a high-value commodity.

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