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INNOVATIVE FOOD PRODUCTS - NUTRACEUTICALS AND FUNCTIONAL FOODS

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Review Based Book Chapter

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REVIEW BASED BOOK CHAPTER

ANTI-DENGUE POTENTIAL OF THE PAPAIN PROTEIN OF CARICA PAPAYA

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Abstract

Dengue hemorrhagic fever (DHF) kills estimated 390 million people yearly throughout the world, according to the WHO in 2019. The Aedes aegypti mosquito, which carries the dengue virus, is the source of this illness. Thrombocytopenia and dehydration are two clinical signs of this illness to be concerned about. There are currently only two treatment approaches: fluid replacement and symptomatic therapy. Consequently, a therapeutic that can get around this restriction is needed. The thrombocyte count rises because the papain in Carica papaya L. can stimulate the release of thrombocytic cytokines including IL-6 and thrombopoietin. Megakaryocyte and thrombocyte production can both rise with the expression of ALOX 12, which papain can do. Using the aqueous two-phase system and the TPP (Three Phase Partitioning) system for extraction process, papain, extracted from Carica papaya L. Papain may be effective in treating dengue fever and restoring normal platelet counts. Further in-depth studies can suggest the dosage and potential adverse effects of papain drug for treating dengue fever.

Keywords
Dengue Fever, Anti-dengue, Papain, Carica Papaya, Cytokines, Protein Purification

Introduction

Carica papaya

The tree like herbaceous plant that belongs to the family is known more widely as papaya. Its scientific name is Carica papaya [1]. A member of the caricaceae family, C. papaya is widely distributed in the tropics and subtropics [2]. Over 6.8 million tonnes of the fruit are produced annually worldwide, as reported by the Food and Agriculture Organization of the United Nations (FAO) [3]. The C. papaya plant, which is well-known for its usage in traditional remedies, was discovered to be the most effective anti-dengue agent. The papaya fruit also contains riboflavin, folate, and niacin in addition to vitamin C, vitamin A, vitamin E, vitamin K, vitamin B 12, and vitamin B6. Macro and micronutrients including salt, potassium, magnesium, iron, copper, and zinc may all be found in plenty. There are many bioactive chemicals, and the primary source is thought to be medicinal plants. A wide variety of insecticides, microbicides, and medicinal medications are derived from secondary metabolites such alkaloids, tannins, proteins, carbohydrates, terpenoids, steroids, and flavonoids [4]. Enzymes such as papain and chymopapain can be found in abundance in papaya leaves [2].

Papain

Latex from the papaya (Carica papaya L.) plant is the source of papain (EC 3.4.22.2), an endolytic plant cysteine protease enzyme [5]. Most cysteine proteases belong to the papain family [6]. Papain is extracted by slicing the peel off of an unripe papaya and drying the latex that drains out. Papain's activity increases as fruit ripens, so choose fruit that still has some green on it. As a proteolytic enzyme, papain plays a fundamental role in many essential biological processes in all organisms and is a member of the papain superfamily [5]. Wurtz and Brochut coined the word papain to describe the proteolytic principle found in papaya latex [7]. Important features of papain like molecular weight, number of amino acids, optimum temperature, optimum PH, binding affinity etc. are describe in Table 1.

Structure

In proteins and polypeptides, it acts as a sulfhydryl protease, hydrolyzing the carboxyl terminus of arginine and lysine. It works in a wide pH range (5-8) and is stable even at higher temperatures (65°C) [8]. The structure of papain is given in Figure 1.

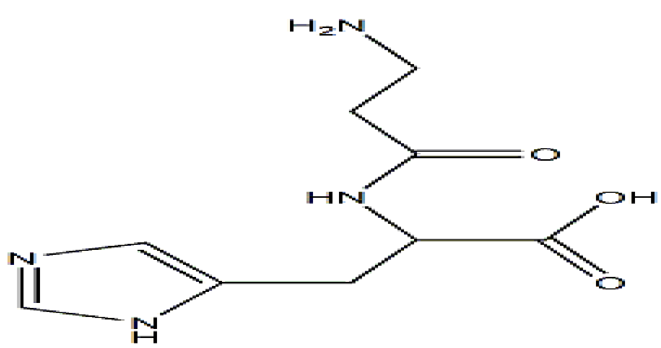

Figure 1. Structure of papain

Table 1. Features of papain

No. of amino acids	212 [9]
Molecular formula of papain	$C_{19}H_{29}N_7O_6$
Molecular weight of papain	23KDa
Optimum temperature	50°C - 59°C [10]
Optimum PH	pH 4.5 – 6.6 [10]
Co factor	Mg2+ [10]
Catalytic efficiency (Kcat/Km)	59.776 - pmL/mg.min [7]
Binding affinity	(Km - 0.83 mg/mL) [7]

Extraction of Papain from Papaya Latex

Fruits contain a fluid called latex. The nutrients in this latex promote in fruit development and ripening. The papain enzyme is found in the latex of papaya fruits, which is harvested by cutting or incising the fruits with a sharp stainless-steel device. It's important to keep a few things in mind while collecting latex from papaya fruit: the incision shouldn't be deeper than 2 mm, the latex shouldn't be soaked in water, and the juices shouldn't be combined with it otherwise the quality of the crude papain enzyme will be compromised. As latex dries quickly following an incision, it's important to collect it as soon as possible and keep an eye out for any foreign debris or contamination as you do so. It is recommended to add 0.3M NaOH to latex before storing it to prevent oxidation. Papaya latex is also sold as a spray-dried form on the marketplace [11].

Papain Isolation and Purification

It is critical to purify and isolate papain from fresh latex in its native crystalline form. The aqueous two-phase system and the TPP (Three Phase Partitioning) system have been reported as common methods for the isolation and purification of papain. TPP, on the other hand, is a simple, low-cost, scalable technique that can be used directly with crude suspensions [12].

The Aqueous Two-phase System (ATPS)

To fulfil these requirements, the aqueous two-phase system (ATPS) might be used. The inexpensive material prices, quick processing time, minimal energy requirements, and low denaturation of proteins are the primary reasons for this. By combining clarification, concentration, and purification of the target product into a single unit operation, aqueous two-phase systems (ATPS) have demonstrated their utility in downstream protein processing. These systems typically comprise two polymers or a single polymer and a salt solution in water (Figure 2). ATPS has been used effectively for enzyme separation and purification at high throughput. When employing such systems, the desired protein partition may be achieved by adjusting a variety of processing parameters. The cheap cost and low viscosity of polymer-salt water systems make them preferable to polymer-polymer-water and polymer-polymer-salt-water systems. Among the latter, the polyethylene glycol (PEG)-phosphate system describe in Figure 2 is the most popular, while other multivalent anion salts have proven helpful as well [13].

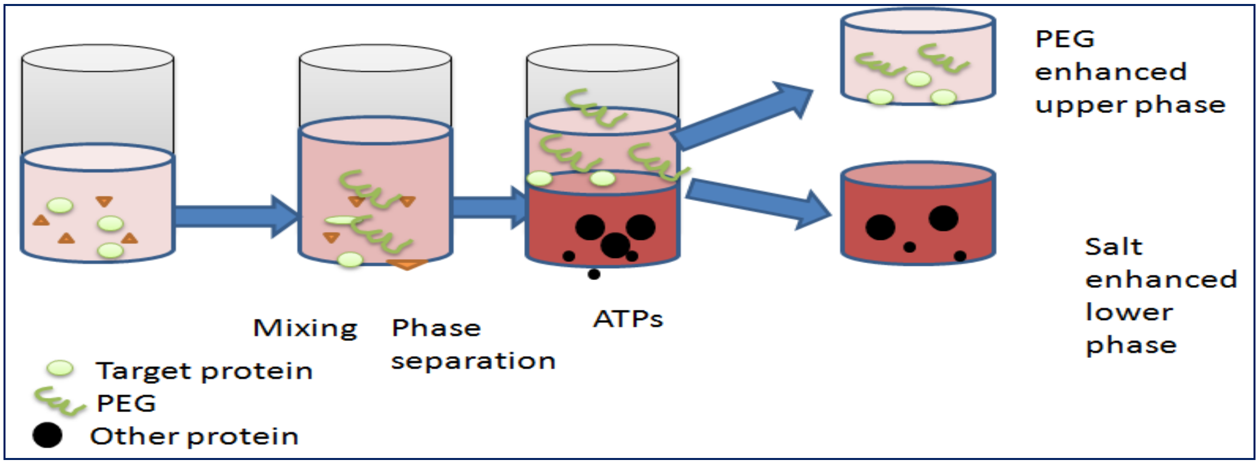

Figure 2. The Aqueous Two-phase System (ATPS)

Three Phase Partitioning (TPP)

Aqueous separation technologies like Three Phase Partitioning (TPP) have been successfully used to extract enzymes from a wide range of biological fluids, tissues, and cells, including those of plants, animals, and microbes, as well as from common surroundings and fermentation broths. TPP was initially reported by Lovrien's lab and is now widely utilized to purify a variety of target macromolecules for a variety of purposes as a result of its versatility and status as an early-exploratory technique. According to a study of the relevant literature, over 70% of TPP-based investigations have concentrated on protein and enzyme recovery, while the remaining 30% have dealt with oils, lipids, small-molecule chemical substances, DNA, and carbohydrates.

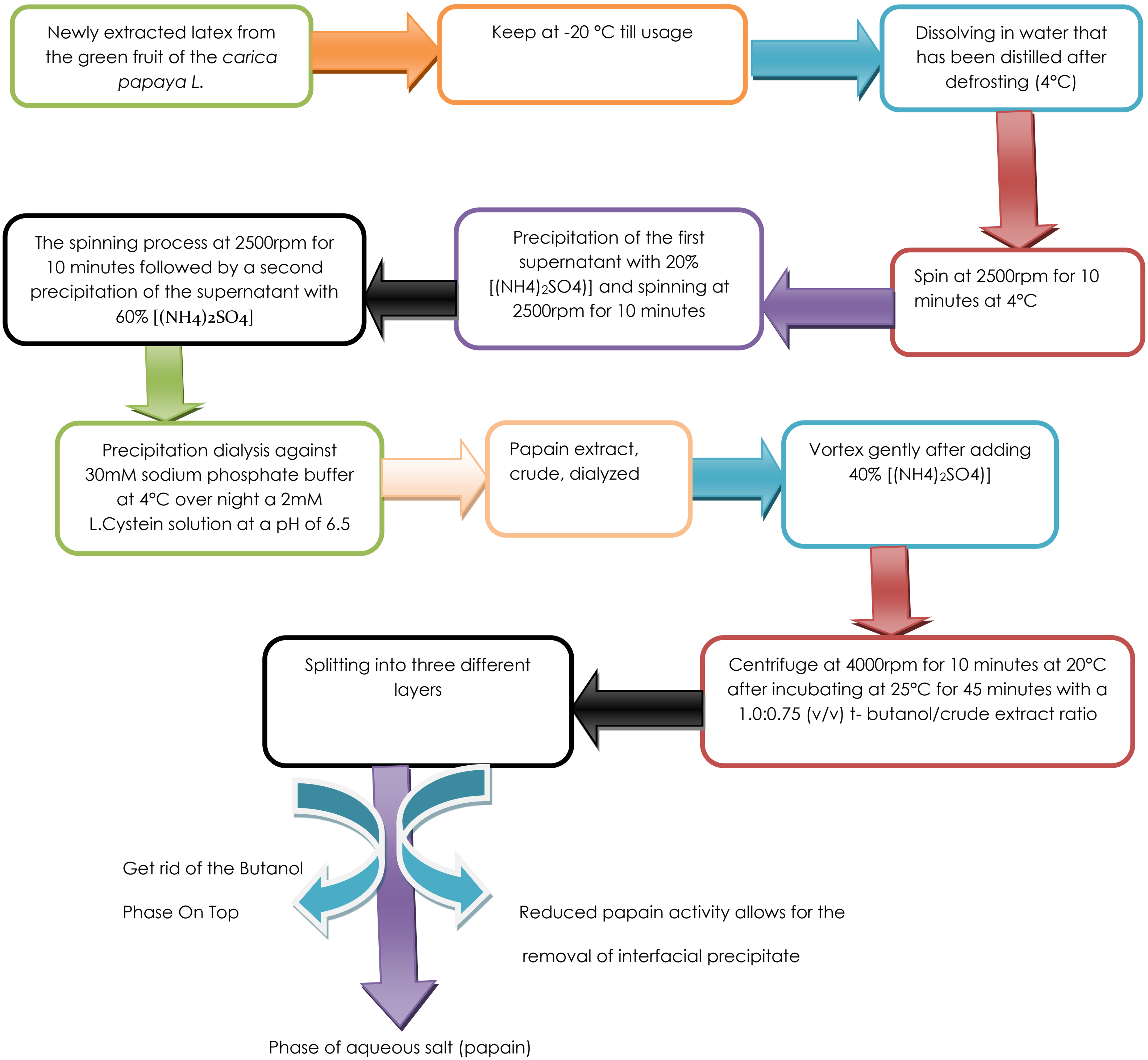

Figure 3. Process employing the Three Phase Partitioning technique that highlights the key steps used to extract papain from the raw papaya latex

The TPP was characterized as a three-stage recovery batch process depicted in Figure 3, combines alcohol precipitations with salting out to extract, dewater, purify, and concentrate the proteins of interest for use in either small- or large-scale production settings. The basic premise is to combine the crude slurry (protein extract) with a solid salt, often ammonium sulphate [$(NH4)_2SO4$], and an organic solvent, typically t-BuOH (tert-butanol), to form a three-phase mixture shown in Figure 3. This method relies on the salt's capacity to partition an otherwise miscible t-BuOH-water combination into an organic-rich upper phase and a water-rich lower phase. Proteins provide a protein layer in which the desired macromolecule can precipitate and be readily removed. The mechanisms of salting out, isoionic precipitation, cosolvent precipitation, osmolytic, kosmotropic precipitation, protein hydration changes, and conformation tightening all work together in a complex interaction way to recover the target protein throughout the TPP process. TPP was employed to isolate many types of enzymes since it is effective at doing so, as seen by the observed rise in overall activity. A wide variety of proteases have been isolated and purified, including ficain, zingibain, cucumisin, papain from dried papaya peels, alkaline proteases from farmed giant catfish, proteinase, and calotropain from Calotropis procera latex, and many more [14].

Importance of Papain

Papain has attracted a lot of attention because of its remarkable properties, which include high enzymatic activity, a broad pH range of action, high-temperature stability, low cost, and non-toxicity. The US Food and Drug Administration (FDA) has acknowledged this enzyme preparation as a generally recognized as safe (GRAS) chemical [5, 6] and it has been put to use in the dietary sector to create bio-peptides by hydrolysis of food proteins [3, 4]. Consequently, papain was utilized as a food ingredient in the meat tenderization, baking, and milk industries [5, 6]. Papain is an enzyme having antibacterial, anti-inflammatory, and debridement properties that has been shown to enhance tissue regeneration when used in biomedical therapeutic settings. Wound healing ointments and gels containing this ingredient are currently commercially available [8–10]. Papain is one of the probable active components, and it has been shown to reduce the scar and improve the cosmetic appearance of the treated area by encouraging local cell multiplication through the increased production of cytokines [11]. Figure 4 shows the use of papain in different industries.

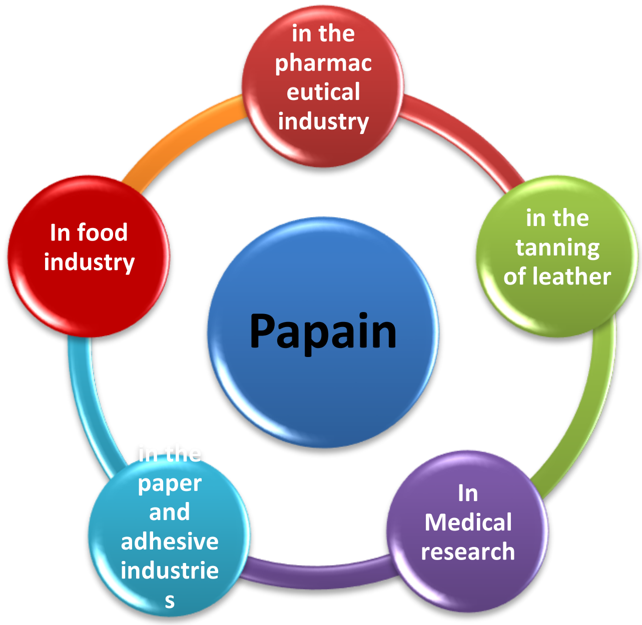

Figure 4. Importance of papain in different industries

Dengue Fever

After malaria, dengue fever is the second most prevalent human illness spread by mosquitoes [15]. Dengue fever, an infection caused by the dengue virus and carried mostly by female Aedes aegypti mosquitoes, is a serious and sometimes fatal disease [16]. Dengue fever is caused by a virus in the family Flaviviridae that is spread via the bite of an infected mosquito [17]. Severe instances of this viral illness were reportedly first discovered in 1950 during an outbreak in Thailand and the Philippines. It has now been discovered in several other countries. The economic and public health consequences of dengue's rapid worldwide expansion as a result of rising global temperatures are already being felt. In 2021, the epidemic of dengue fever reaches the countries of Reunion Island, Peru, Paraguay, Kenya, Fiji, Colombia, the Cook Islands, and Brazil. Dengue fever can cause severe debilitating symptoms such as severe weakness, extreme malaise, red eyes, red face, vomiting, discomfort behind the eyes, nausea, muscle aches, skin rash, high temperature, back pain, and even death. The mosquito becomes infected by sucking blood from a person who already has the virus. Viruses can remain dormant in vector mosquitoes for years after infection, and in both circumstances, vertical transfer is extremely rare [16].

Epidemiology

Recent years have seen a rise in the occurrence of dengue fever over the world, putting almost half of humanity at risk. Over 80% of the 100-400 million annual illnesses are considered moderate or asymptomatic. Because of this, dengue instances are frequently underreported. Additionally, many instances are misdiagnosed. One modeling estimate places the number of dengue virus infections at 390 million per year (95% confidence interval: 284-528 million), of which 96 million (range: 67–136 million) show clinical symptoms of the illness. While there is a potential for infection in 129 different nations, the real impact is concentrated in Asia (70%) rather than elsewhere. World Health Organization data show that in the last two decades, the number of dengue cases has increased from 505,430 in 2000 across over 2.4 million in the year 2010 and 5.2 million in 2019 worldwide. There was a dramatic rise in deaths among young people between 2000 and 2015, with the number of reported deaths going from 960 to 4032 during that time period. In both 2020 and 2021, there appeared to be a decline in both the overall number of cases and the number of recorded deaths [15].

Population growth, unchecked urbanization in tropical and subtropical areas with poor sanitation, unreliable water supply systems, poor solid waste disposal, an increase in non-biodegradable containers in endemic areas leading to the proliferation of Aedes mosquito breeding sites, a lack of effective mosquito control programs, and human travel are all potential causes of the global resurgence and spread of dengue fever. The fast dissemination of the illness may also be attributed to the rapid development of dengue viruses with much more virulent genotypes. Every year, about 500,000 individuals with severe dengue need treatment; a significant percentage of these patients are children. Nearly 5% of those afflicted pass away. The yearly number of patients expected to die is 25,000 [15].

Treatment

Numerous studies have been conducted to create a vaccine against dengue viral illness because of its increasing importance in recent decades. However, there is currently no 100% efficient vaccine on the market, though some are available in various locations. In order to eradicate this viral infection, researchers have relied on optimal control and management strategies [15]. The possible mode of action of such enzymes show that these are useful for enhancing the blood platelet count and blood clotting factor in human [2].

The bone marrow microenvironment is essential for the formation of platelets, which are cellular effectors of mammalian hemostasis. Thrombopoietin (TPO) is principally responsible for controlling the varied and intricate process that produces platelets. A synergistic role is also played in the release of platelets from megakaryocytes by other cytokines, including interleukin-1 (IL-1), IL-3, and IL-6, stem cell factors, fibroblast growth factor (FGF), and erythropoietin (EPO). A powerful phospholipid mediator called Platelet Activating Factor (PAF) also has an impact on platelet aggregation and degranulation.

The Papain Mechanism in the Hemorrhagic Fever of Dengue

The ability of papain to stimulate thrombocytes' production of cytokines like IL-6 is its mechanism of action in dengue hemorrhagic fever is depicted in Figure 5. According to Aziz et al. [18] secretion of this kind is carried out by mesenchymal stem cells, such as SHED, and haematopoietic stem cells. It will directly promote proliferation, maturation, and stimulation platelet production by increasing the secretion of thrombopoietin in the liver, leading to an increased platelet count. Mast cell proteases, tryptases, and chymase, which can cause plasma leakage, can also be broken down by the proteolytic enzyme papain (Figure 5). RNA analysis of study participants by Subenthiran et al. [19] reveals the presence of Arachidonate 12-lipoxygenase (ALOX12) and Platelet-Activating Factor Receptors (PTAFR) genes. At the conclusion of the third day of this investigation, the ALOX12 gene had grown by 15-fold; it is known that these genes are associated with enhanced megakaryocyte formation, followed by platelet creation, via the 12- Hydroxyeicosatetraenoic acid, as well as 12-HETE. The target of RUNX1 transcription, which controls the expression of haemopoietic-specific genes in megakaryocytes and platelets, is known to be ALOX12. Despite this, direct connections between DENV and ALOX12/PTAFR are not mentioned by a number of authors.

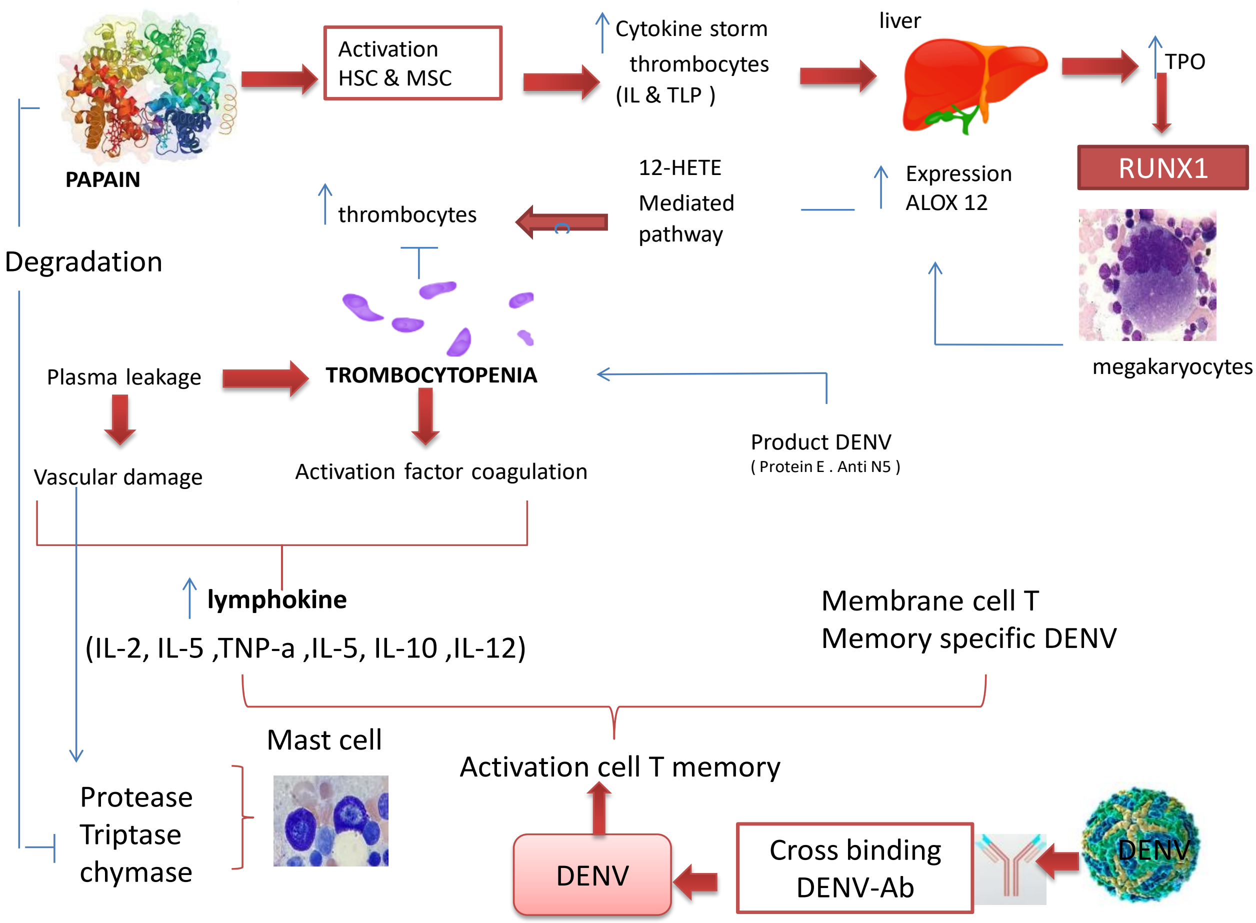

Figure 5. Mechanism of papain to enhance the platelets count

Conclusion

Papain has great potential for treating dengue fever and restoring normal platelet counts. Papain stimulates thrombocytes' production of cytokines. Three phase partitioning method of Papain enzyme purification is more suitable for research purpose. Papain's structure, isolation and function in dengue fever have been reviewed well and will help in the future studies linked with drug design to treat dengue fever.

Conflicts of Interest

The authors declared no conflict of interest.

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