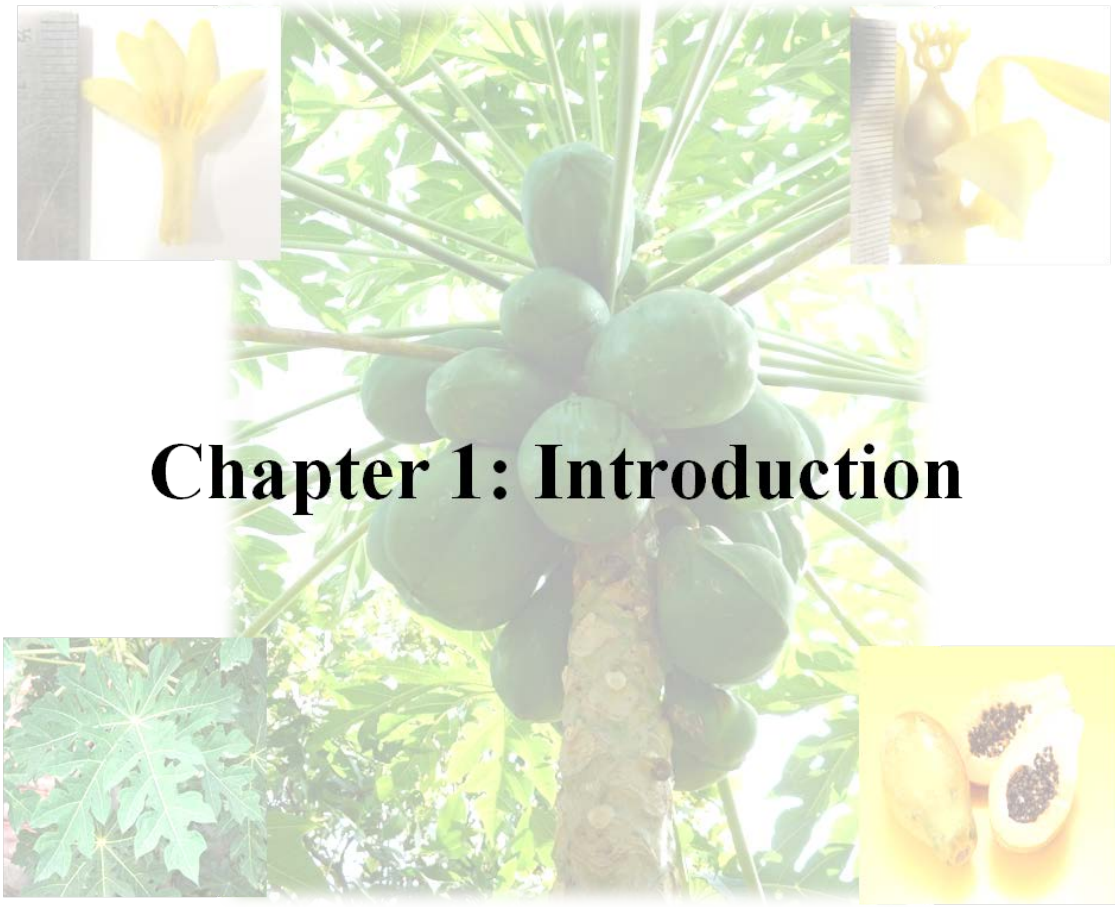

Chapter 1: Introduction

1.1 Introduction

Platelet is one of the important components of blood whose function (along with coagulation factors) is to stop bleeding by clumping and clotting the injured blood vessel [1]. Platelets, also called thrombocytes (thromb + cyte, "blood clot cell"), are anucleated, and derived from the megakaryocytes of the bone marrow. In every human being each day about $1x10^{11}$ platelets are produced by cytoplasmic fragmentation of megakaryocytes [2]. They are 2,50,000 to 4,00,000 /µL in human blood [2], [3] with life span of 8 to 14 days [4], [5] and are destroyed in the spleen [3]. There are many diseases associated with abnormality of platelets, thrombocytopenia is one of them. The condition of platelet counts less than 1,50,000 /µL is termed as 'thrombocytopenia'. Many diseases (cancer, aplastic anemia, viral or bacterial infection, sepsis), surgeries and drugs (sulfonamide, rifampicin) [6]–[8] can cause thrombocytopenia [9]. Some auto-immune mechanism with the production of auto-antibodies against platelets may result in the destruction of platelets via reticuloendothelial system and cause idiopathic thrombocytopenic purpura (ITP). The resulting symptoms are often dramatic and associated with a variety of hemorrhagic sequelae, including epistaxis, petechiae, gastrointestinal bleeding and intracranial hemorrhage.

The line of treatment for thrombocytopenia includes use of corticosteroids, immunoglobulins or splenectomy, however it is not effective for 25 to 30% of patients with chronic ITP [10], [11]. Currently, no feasible alternative therapy is available to treat chronic ITP and thus fatal hemorrhage occurs in approximately 16% of the affected patients [12]. Despite of significant need for the development of new therapies for ITP, little progress has been made since the discovery of Intravenous Immunoglobulin (IVIG) therapy in 1981 [13], [14].

Under such conditions, traditional medicine is the default option. The failure of allopathic armamentarium has channelized efforts into rediscovering the wealth of traditional and complementary medicines (T&CM) [15].

While looking for effective traditional remedy for thrombocytopenia in literature, a large number of blogs describing use of *Carica papaya* for the treatment of dengue fever were seen on the social network. Dharmarathna *et al*, have described the enhancement of platelets in rats on administration of decoction of *Carica papaya* leaves [16]. *Carica papaya* leaves are traditionally used for the treatment of dengue and other ailments in Malaysia [17], [18]. Few patents on application of dry *Carica papaya* leaves as platelet booster have been filed [19], [20].

Inspired by these facts, an attempt was made to conduct a systematic research on an ethanopharmacologically important plant (*Carica papaya*) with a view to rectify the existing lacunae.

1.1.1 *Carica papaya* Linn. Origin and Distribution

Carica papaya Linn. is indigenous to tropical America [21]. It is a popular and economically important fruit tree of tropical and subtropical countries. *Carica papaya* belongs to Caricaceae family. It is a fast-growing, semi-woody tropical perennial herb. It is a polygamy species i.e. can be male, female or hermaphrodite.

Figure 1.1: *Carica papaya* plant

1.1.2 Taxonomy of *Carica papaya*

Table 1.1: Taxonomical classification of *Carica papaya* [22]

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae – either polypetalous or gamopetalous corollas and often with ovules attached to the walls of the ovary
Order	Violales – compound ovary and mostly parietal placentation
Family	Caricaceae – Papaya family
Genus	*Carica*
Species	*Carica papaya* L. – papaya

1.1.3 Morphology of *Carica papaya* plant

Leaves: Papaya plants have large palmately compound leaves with 5-10 pinnate lobes of various widths (20–60 cm). Dorsoventrally flattened leaves have a long hollow petioles arranged spirally upto 1 meter long and clustered at the crown confined to the top of the stem. The leaf, unlike the fruit, is not a source of the protein-dissolving enzyme papain, but present in the latex (sap) of leaf stem [23], [24].

Figure 1.2: leaves of *Carica papaya*

Carpaine, pseudocarpaine and dehydrocarpaine I and II, choline, carposide, vitamin C and E have been reported in *Carica papaya* leaves [25], [26].

Figure 1.3: Stem of *Carica papaya*

Stem: Diameters of adult *Carica papaya* plants vary from 10 to 30 cm that is marked by scars of fallen leaves. Papaya exhibits strong apical dominance with rare branching unless the apical meristem is removed, or damaged [27].

The presence of β-Sitosterol, glucose, fructose, sucrose, galactose and xylitol have been reported in *Carica papaya* stem [25], [26].

Roots: The papaya root belongs to adventitious root types which are predominately a non-axial, fibrous, and composed of one or two 0.5–1.0 m long tap roots. Secondary roots emerge profusely from the upper sections and branch. Healthy roots are whitish cream colored with no laticifers [28].

Figure 1.4: Roots of *Carica papaya*

The root mainly contains carposide and myrosin enzyme [25], [26].

Flowers: Papaya flowers are produced profusely near the trunk apex. Individual flower longevity may be of 3–4 days. Papaya flowers are actinomorphic cymes arranged in inflorescences on the leaf-stem junction.

Figure 1.5 (A): Actinomorphic cymes arranged in inflorescences on the leaf-stem junction. **1.5 (B):** Male flower of *Carica papaya*

Male (staminate) flowers: Male plants produce very long inflorescences that contain dozens or even hundreds of flowers. Male flowers are pendent shaped and sessile with

small cup shaped calyx having 4-5 tooth. The stamens are in 5 pairs; exist in 2 whorls alternate with the petal lobes. The 3.5 cm long, light yellow colored, trumpet shaped corolla with 5 spreading lobes usually borne on a long flower stalk includes yellow pollens on anthers, tiny nonfunctional ovary and unbranched stigma [29], [30].

Figure 1.6 (A): Female flower of *Carica papaya.* **1.6 (B):** Longitudinal section of female papaya.

Female flowers: The arrangement of female flowers is either solitary or in cymes of a variable number of flowers (2 to 15). The corolla is yellow in color, fleshy, twisted and lanceolate shaped with free petals. The cup shaped calyx is 3-4 mm long with 5 narrow teeth. The ovary is round, superior and consists of many ovules along with cavity at the centre. Fan shaped, sessile stigma is present with deeply-cleft [29].

There are 2 types of **hermaphrodite flowers** present in papaya plant; elongata and pentandria. Elongata flowers are in short-peduncled cluster with semi-united petals. The stamens are present in pairs with elongated ovary.
Pentandria flowers are similar to female flowers with additional 5 stamens [31].

Figure 1.7 (A): Unripe fruit of *Carica papaya.* **1.7 (B):** Ripe fruit of *Carica papaya*

Fruits: The fruits are melon-like, oval to nearly round, somewhat pyriform or elongated club-shaped, 15-50 cm long and 10-20 cm thick, weighing up to 9 kg. The skin is waxy and thin but fairly tough. When the fruits are immature, it is rich in white latex and the skin is green and hard. As ripening progresses, papaya fruits develop a light or deep yellow-orange colored skin while the thick wall of succulent flesh becomes aromatic, yellow orange to various shades of salmon or red, juicy, sweetish and somewhat like a cantaloupe in flavor but some types are quite musky [32], [33].

Papaya fruit is reported to be rich in phytochemicals like thiamine, riboflavin, niacin, and carotene, citric and malic acids (green fruits), linalool, benzylisothiocyanate, cis and trans 2,6-dimethyl-3,6-epoxy-7-octen-2-ol, carpaine, benzyl-β-D-glucoside, 2-phenylethyl-β-D-glucoside, 4-hydroxyphenyl-2-ethyl-β-D-glucoside and lipids [25], [26].

Seeds: The seeds are black or greyish, spherical with 5 mm diameter. The seeds are attached to inner wall of ovary in 4-5 rows having gelatinous sarcotesta coating.

Carpaine, benzylisothiocyanate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β-sitosterol, caricin and an enzyme myrosin are reported to be present in papaya seeds [25], [26].

1.1.4 Pharmacological functions of *Carica papaya* Linn.

1.1.4.1 Traditional Formulations

In many countries different formulations of papaya been used to cure diseases as listed below.

In Maldives decoction of plant leaves been used to expel worms from stomach. Boiled extracts of the stem bark are usually given for urinary problems. Inner bark decoction

is useful to soothe toothache by oral consumption while the stem bark paste can be applied to boils, burns and wounds to facilitate the healing process [34].

In Madagascar, *Carica papaya* leaves tea been used in order to treat gastric ulcers as well as general gastric discomfort [35].

In Congo the dried leaves are inhaled to treat bronchial asthma [36]. In rare circumstances, the infusion of the flowers is used for its emmenagogue, febrifuge and pectoral properties [37].

In Sierra Leone, the latex sap is drunk raw to treat jaundice. In DR Congo and Senegal, three to four spoonfuls of the latex sap is taken empty stomach to treat worm infestations [36].

In Indian traditional medicine back in 16th century, the unripe and green fruit was made into curry and eaten by woman to stimulate secretion of milk.

Papaya fruits are extensively used in day today life due to many nutritional benefits as well as medicinal values.

Papain and chymopapain, two important enzymes of papaya plant got FDA approval in 1983 for Chemonucleolysis treatment (Chemonucleolysis is a treatment in which an enzyme, chymopapain, is injected directly into a herniated lumbar disc which results into dissolution of material around the disc, thus reducing pressure and pain) however in 2003 the supply of chymopapain was discontinue [38]–[40]. Papain and chymopapain both belong to proteolytic enzyme class (enzymes that dissolve proteins) [41], [42]. The latex of *Carica papaya* is a rich source of four cysteine endopeptidases papain, chymopapain, glycyl endopeptidase, and caricain [40], [43]. The structure of papain and chymopapain was identified by Drenth *et al* and Jansen *et al* respectively [44], [45].

1.1.4.2 Preclinical Investigations

The preclinical investigations on efficacy of *Carica papaya* for various ailments carried out by many groups are briefly summarized below:

Tan *et al* have reported that the methanolic extract of *Carica papaya* leaf (50 µg/mL) to be non toxic towards HepG2 cell growth [46].

The acute toxicity study of freeze dried *Carica papaya* leaves aqueous extract on female Sprague Dawley rats reveled that LD_{50} > 2000 mg/kg showed no toxic effect with any significant abnormality in necropsy [47], [48].

A significant ($p < 0.05$) decrease in serum glucose level, along with reduced alanine aminotranferase, aspartate aminotransferase and alkaline phosphatase levels and improved plasma insulin levels on administration of aqueous extract of *Carica papaya* leaves to diabetic Wistar rat model has been reported [49]–[51].

Administration of ethanolic extract of *Carica papaya* leaves (50 and 200 mg/kg BW) to lead-induced stressed male albino Sprague Dawley rats (160-180 g) have been reported to show antioxidant activity by significantly ($p < 0.05$) increased glutathione content (GC) and reduced protein carbonyl content (PCC) in the femur of the sacrificed rats [52].

Tan *et al* have studied the hepatoprotective activity of hydroalcoholic (80%) extract of *Carica papaya* leaves (0.5 µg/mL) on oxidative stress induced human liver cells (HepG2 cells) and reported significant ($p < 0.05$) reduction of the reactive oxygen species (40%) and increased glutathione peroxidase activity (glutathione content: 1.75 nmol/mg protein) compared to the controls [46].

The treatment of gastric ulcer induced Sprague Dawley rats with aqueous extract of *Carica papaya* leaves have revealed significant ($p < 0.05$) increase in glutathione

peroxidase levels with decreased malondialdehyde levels and small mean ulcer index compared to ethanol-induced control group [53].

Imega *et al* have studied the antisickling properties of hydroalcoholic extract of *Carica papaya* leaves. The extract showed inhibition in the formation of sickle erythrocytes (SS cells) compared to untreated SS cells (60% of SS cells). The extract also decreased the percentage haemolysis of SS cells compared to untreated SS cells [54].

Lohiya *et al* have studied the contraceptive effect of chloroform, methanol and ethyl acetate extract of *Carica papaya* seeds on male albino rats which showed decrease in the total body weight as well as weights of their reproductive organs (testis, epididymis, seminal vesicle and ventral prostate) compared to control. Furthermore the sperm motility was totally inhibited with significant declined in sperm viability and sperm density [55].

BioRex gel prepared by Mikhal'chik *et al* from fermented papaya showed accelerated wound healing and reduced wound area compared to control rats and reduced severity of local inflammation in rats with burn wounds [50].

Topical application of aqueous extract of *Carica papaya* leaves twice a day on dorsal neck excision wound of male Sprague Dawley rats have showed decreased healing time compared to Vaseline-treated control group [56].

An inhibited growth of *Escherichia coli* (Inhibition zone = 8.30 mm), *Micrococcus luteus* (8.23 mm), *Pseudomonas aeruginosa* (8.23 mm), *Bacillus cereus* (9.20 mm), *Klebsiella pneumonia* (6.17 mm) and *Staphylococcus aureus* (8.20 mm) have been reported on application of alcoholic extract of papaya leaves, and is significant compared to ciprofloxacin [*E. coli* (23.50 mm), *M. luteus* (16.97 mm), *P. aeruginosa* (25.00 mm), *B. cereus* (21.83 mm), *K. pneumonia* (14.83 mm) and *S. aureus* (29.83 mm)] [57].

Dawkins *et al* have studied the antibacterial activity of *Carica papaya* seed extracts against gram positive and gram negative organisms which showed the most inhibition zones of *B. cereus* followed by *E. coli, Streptococcus faecalis, S. aureus, Proteus vulgaris* and *Shigella flexneri* [58].

Studies of antibacterial activity towards *S. aureus, E. coli, and P. aeruginosa* of different extracts of *Carica papaya* leaves *viz* petroleum ether, 1% HCl, acetone, ethanol, and its aqueous extract have shown Petroleum ether extract to be the most effective compared to standard antibiotic drugs perflacin and cefuroxime for all bacteria followed by ethanol extract, 1% HCl extract and acetone extract. Aqueous extract been reported to inhibit *S. aureus* and *E. coli* only [59].

Bhaskaran *et al* have reported well diffusion method to study the antifungal effect of ethanol extract of *Carica papaya* leaves which showed inhibited growth of *Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis, Cryptococcus neoformans* and *Candida kefyr* compared to ketoconazole [57].

Oral administration of methanolic extract of *Carica papaya* leaves to malaria induced Swiss albino mice have been reported to reduce parasitemia levels from (Day-1:66% to Day-5:12%) of treatment compared to untreated control (Day-1:66%, Day-5:80%) [60].

Patil *et al* have studied the effect of aqueous extract of *Carica papaya* leaves on blood platelet count against cyclophosphamide induced thrombocytopenic Wistar rats for duration of 15 days. The significantly ($p < 0.001$) increased the platelet count compared to cyclophosphamide treated control group have been reported [61].

1.1.4.3 Data on clinical investigations

1.1.4.3.1 Dengue Fever

Recovery of platelet counts, white blood cells and neutrophils to its normal levels have been observed by Ahmed *et al* in a 45-year old male patient during diagnosis

with dengue fever, on administration of *Carica papaya* leave extracts (orally, twice daily for five consecutive days with sucrose) [62].

Yunita *et al* have studied the effect of *Carica papaya* leaves extract capsules (CPC) on 80 patients suffering from dengue fever. Two CPC were administered daily three times while the control group received a standard treatment. A significant ($p<0.05$) increase in the platelet count in the patients treated with CPC compared to control group was observed. [63].

1.1.4.3.2 Burn Wound Infection

Starley *et al* have observed that daily use of mashed *Carica papaya* pulp to full thickness and infected burns results into effective desloughing of necrotic tissue, preventing burn wound infection, and providing a granulating wound suitable for the application of a split-thickness skin graft (STSG) [64].

1.1.4.3.3 Chemonucleolysis

Decrease of root pain (up to 85%) of 80 patients suffering due to herniated discs has been reported after injection of proteolytic enzymes present in *Carica papaya* into the lumbar intervertebral disc [65].

1.1.4.3.4 Human Intestinal Parasitosis

An elixir composed with air-dried *Carica papaya* seeds and honey (CPH) or honey alone (placebo) were administered to 60 asymptomatic Nigerian children with stool microscopic evidence of intestinal parasites. No parasites were detected in the stools of the children given CPH elixir than those given only honey [66].

1.2 Rationale of the research work

The World health organization (WHO) has recognized important contribution of the traditional medicine to provide essential healthcare. Fifty-sixth World Health Assembly in May 2003 passed the resolution WHA 56.31 on traditional medicine which was an initiative to enhance safety, efficacy and quality of different systems of traditional medicine. As a result, many countries including India have established, or initiated the process of establishing national regulations regarding herbal medicines. Under the leadership of Dr. Margaret Chan (Director-General of WHO Traditional Medicine Strategy), new resolution WHA 62.13 on traditional medicine focusing on the following objectives been conceded.

1) Formulating national policies for building the knowledge base on traditional medicines.
2) Strengthening safety, quality and effectiveness through regulation.
3) Promoting universal health coverage by integrating T&CM services and self-health care into national health systems.

A systematic research to explore the available wealth of traditional knowledge is demand of the day.

The unsurpassed success story of the constant systematic research in Chinese herbal medicine is the discovery of artemisinin and dihydroartemisinin, by Tu Youyou and her group to treat malaria. Before 1960s half a million people used to die of malaria every year. During the 1960s and 70s, Tu Youyou carried out extensive survey of literature on the ancient Chinese herbs and discovered that various preparations of *Artemisia annua* (sweet wormwood) plant can be used against fever. She started the use of plant on the malaria-infected mice as a major Chinese project. The plant showed excellent potency against malaria. She isolated the bioactive component and identified as artemisinin using bioassay guided fractionation technique. The discovery of artemisinin saved the lives of millions of people and the mortality rate by malaria

decreased rapidly during the past 15 years. The systematic approach has saved millions of lives [67] and the efforts made by Nobel laureate Tu Youyou been recognized worldwide.

1.3 Objective of the work

- To investigate a phytochemical or group of phytochemicals which is responsible for increase in thrombocyte count.
- Bioprospecting among different parts of *Carica papaya*, focusing mainly on different secondary metabolites.

1.4 Methodology of the work

In order to achieve above mentioned objectives, following methodology was applied:

a) Identification of plant and pharmacognostic study.
b) Separation of various phytochemical groups present in *C. papaya*.
c) Preparation of thrombocytopenic model. Bioassay guided fractionation to isolate the compound responsible for antithrombocytopenic activity. Identification of the compound responsible for the bioactivity.
d) Method development for quantitative analysis and bioprospecting of the active component in different parts of *C. papaya*.
e) Screening of antioxidant activity of extracts of different parts of *C. papaya.*

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